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STUDIES ON THE RELATION OF THE EXTERNAL TO THE INTERNAL SECRETION OF THE PANCREAS

I. Biochemical Study on the Nature of the Action of Trypsin on Insulin[1]

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Our present conception of the cause of diabetes is based on the supposition that it is due to a deficiency of the internal secretion of the pancreas (insulin) resulting from a functional or structural change in the islands of Langerhans.

Notwithstanding the current opinion that the external secretion is in no way related to the internal secretion of the pancreas, in the causation of diabetes, there appears to be some ground for a contrary belief. For example, procedures which favor the depletion or exhaustion of the external secretion (such as the use of secretin) are believed to exert a favorable influence on the internal secretion. The genetic relation and the structural proximity of the islands of Langerhans to the zymogenic cells, together with the supposed "destructive" action of trypsin on insulin, suggest the possibility that, under certain conditions, at least, the external secretion may affect the internal one. Of course, if the action of trypsin on insulin were as alleged (1) of a proteolytic nature, the supposition that a reaction of this kind could occur within the gland itself, would at once become untenable. However, the rapid disappearance of insulin from the extirpated pancreas under certain conditions, and the easy recovery of insulin under other conditions, made us believe that the effect of trypsin on insulin is not one of cleavage or destruction.

[1] Read before the American Society for Clinical Investigation, May 5, 1924.

As a first step in our investigation of the relationship of the external secretion of the pancreas to the internal, we deemed it advisable to study the effect of trypsin on insulin, the results of which are reported in this communication.

In previous work with the pancreatic ferments (2), (3) we found that properly purified trypsin possessed two additional functions, namely, that of coagulating milk (a rennet action), and the power to shorten the coagulation time of blood (a thrombokinetic action). The latter function it exhibits both in vitro and in vivo. Therefore, other effects than that of proteolysis could reasonably be expected from trypsin. In the work just referred to we have made other observations which bear on the present investigation: 1, that the rennet action of trypsin cannot be separated from its proteolytic action; 2, that the antitryptic action of serum represents an interference phenomenon, i.e., that serum exerts no destructive effect on trypsin, but merely interferes with its action on the substrate. Under such conditions the trypsin is inert, but can again be recovered from the mixture by appropriate means. 3, That whereas serum inhibits the proteolytic action of trypsin, it does not interfere with its milk-coagulating function; 4, that the purified trypsin is relatively non-toxic, and repeated injections into an animal do not cause anaphylaxis, or produce any abscesses, or other deleterious effects.

Recent experiences with methods of isolation and preparation of insulin, as recorded in the literature (4), seem to suggest that trypsin does not readily destroy insulin. For, if trypsin does destroy insulin, and accomplishes this result by proteolysis, then we should find: 1, that trypsin requires a certain length of time in which to digest insulin; 2, that the digestion should proceed most speedily at an optimum temperature; 3, that it requires a certain optimum reaction for the medium in which digestion can take place; 4, that the reaction must be irreversible; and, 5, that the reaction should be hindered by the presence of serum, by virtue of the antitryptic power which serum possesses.

The insulin used in these experiments was that of Eli Lilly & Co., known as iletin. The trypsin was a specially purified preparation made from Fairchild's pancreatin. The method of purification is as follows:

Sixty grams of pancreatin are dissolved in 1000 cc. of distilled water. The solution is filtered. Eight hundred cubic centimeters of a 5 per cent colloidal iron solution are added slowly with constant stirring. A very massive precipitate develops and is removed by filtration, the trypsin remaining in solution. As the quantity of colloidal iron employed is in excess of that needed for the removal of all precipitable substances, the filtrate contains colloidal iron and is stained brown. Powdered chemically pure $CaCO_3$ is then added to the filtrate and the mixture stirred very thoroughly. The colloidal iron congeals and separates from the solution. The mixture is now filtered. The resulting filtrate is water clear. It contains the trypsin plus a small amount of calcium which goes into solution. (Na_2CO_3 or some other

electrolytic salt may be used for this purpose.) To the filtrate is then added 8 volumes of chemically pure acetone. The mixture is at first turbid. Upon standing in the ice-box for some time, a white flocculent precipitate develops. This step may be accelerated by adding to the mixture a few drops of concentrated NaCl solution. When the precipitate settles out it adheres to the bottom and walls of the container; the clear supernatant fluid is removed by decantation without any loss of the substance. A current of air is then passed through the container in order to remove all traces of acetone. When all the acetone is gone the precipitate is found to be very hygroscopic. It begins to dissolve and coalesce. The residue acquires a dark amber color and is of an oily appearance. The substance is free from amylase and lipase. This material is easily soluble in water and hence can be prepared in any concentration. The watery solution is then passed through a Berkefeld filter and kept in the ice-box.

Such a solution has very remarkable keeping properties. It remains potent for months and does not undergo deterioration when kept under sterile precautions, even at ordinary room temperature. Hence one lot suffices for many experiments, a condition which affords greater uniformity and precision in experimental work of this kind.

The purified trypsin is of an acid character. By using calcium carbonate for the removal of the excess of the colloidal iron some calcium goes into solution, thus bringing the reaction of the solution to a point close to neutrality. The stock solution of trypsin employed in our experiments contained 5 per cent trypsin and had a pH of 7.0 (The pH of the solutions used in our experiments was determined colorimetrically.) Doisy, Somogyi and Shaffer (5) have found that insulin is precipitated out of solution when the pH of the solution is 4.7 to 5.6. In our very first experiments we observed that the addition of our trypsin solution to insulin, in sufficient amount, caused clouding and precipitation. This phenomenon we later ascertained to be the result of a change in the pH incidental to the addtion of trypsin (pH 7.0) to the insulin, and was not caused by any specific action of the trypsin on the insulin. On adding the same amounts of trypsin to varying amounts of insulin we found, furthermore, that the degree of precipitation varied with the amount of insulin present. The precipitation was very prompt and took place irrespective of the temperature of the reacting media. Under like conditions equally rapid precipitation occurred when temperature of the trypsin and insulin was that of the ice-box, that of the room, or that of the thermostat. The precipitate remained insoluble provided the pH remained unchanged. Upon addition of trypsin to insulin in sufficient amount to cause complete precipitation, and the precipitate removed by centrifugalization, the pH of the supernatant fluid was found to be 4.8. Elevating the pH above 6.0 or lowering it below 4.6 causes solution of the precipitate. It might then be said that the precipitation of the insulin, under the conditions described, takes place at a pH which lies between 4.6 and 4.8.

By virtue of the fact that insulin could be removed from the solution (by precipitation) by changing the pH and independently of any action of the trypsin upon it, it was possible to study the combining power of trypsin with insulin, and the effect on the activity of the latter.

In pursuing our problem, two groups of experiments were performed. In the first group, the trypsin was permitted to act on the insulin in vitro. The effect on the insulin was then determined by injecting the mixtures into suitable test animals and studying their reaction to them. In the second group of experiments the action of trypsin on insulin was studied in vivo.

In the first group of experiments we sought to ascertain the following points: 1, the time required for the inactivation of insulin by trypsin; 2, the reaction of the medium in which inactivation takes place; 3, the amount of trypsin necessary to inactivate a unit of insulin; 4, the temperature at which the reaction takes place; 5, the reversibility of the reaction, i.e., can insulin be recovered or reactivated after trypsin has acted upon it? 6, the effect of serum on the reaction between trypsin and insulin.

The first point investigated was the rate of destruction or inactivation of insulin by trypsin. Because of the fact that the addition of even small amounts of trypsin (pH 7.0) caused a change in the pH of the insulin solution, sufficient to induce precipitation, the mode of procedure was as follows: Variable amounts of trypsin were added to insulin, and:

a. The mixtures after brief contact at room temperature were injected into rabbits.

b. The mixtures were centrifuged to remove the precipitate and the supernatant fluid injected into rabbits.

c. The precipitates recovered from *b* were suspended in water or saline solution and injected into rabbits.

d. The precipitates recovered from *b* were suspended in water or saline solution, dissolved by the addition of a little alkali (Na_2CO_3 or NaOH) and injected into rabbits

e. The precipitates recovered from *b* were suspended in water or saline solution and dissolved by the addition of a little acid (HCl).

f. The precipitates recovered from *b* were washed in buffered solutions (pH 5.2), the supernatant washings removed, the precipitate dissolved in alkaline solution and injected into rabbits.

g. Same as *f* except that the precipitate was dissolved in acid.

After injection of these various preparations into rabbits, the effect on the blood sugar and the conduct of the animals was observed. In the earlier experiments the dosage of insulin was relatively small and the effect on the animals was studied through the medium of the blood sugar. In the later experiments, however, very large doses of insulin were employed and the blood sugar determinations in most instances were omitted,

because we found that 15 units of insulin invariably produced the characteristic hypoglycemia and convulsions within two hours in fasted animals under 2 kgm. weight. Therefore the occurrence or non-occurrence of convulsions was taken as indicative of insulin activity or its absence.

By mixing trypsin with insulin, and injecting the mixture after very brief contact into fasted rabbits of suitable weight no insulin effects are produced. Neither the temperature of the two agents (trypsin or insulin), nor the amount of insulin used affect the negative result on test animals provided, of course, that enough trypsin is added to the insulin. In certain of the experiments, amounts of insulin exceeding 50 units were mixed with trypsin and injected into rabbits without producing any effect on the animals. The same results are obtained when the mixtures are made at temperatures ranging from 3° to 37.5°C.

When the precipitate which forms upon the addition of our trypsin to insulin, is separated by centrifugalization, and both the supernatant fluid as well as the precipitate (suspended in saline) are injected into rabbits, no physiological insulin effects are produced. We must conclude from such experiments that precipitation of the insulin which results simply from a change in the pH does not protect it from the action of the trypsin, and that enough trypsin attaches itself to the insulin to cause complete inactivation of the latter (protocol 1).

That this is actually the case is confirmed by the following experiment (see protocol 2), in which the precipitate which formed upon the addition of trypsin to insulin was separated by centrifugalization, and washed in several changes of water and then injected into rabbits. The result obtained shows that the insulin has been completely inactivated by the procedure. Evidently enough trypsin combines with the insulin even in this brief contact of the two reagents to cause inactivation of the insulin.

Several questions naturally arise here: 1, What is the nature of the reaction between trypsin and insulin which results in the inactivation of the insulin? 2, What becomes of the insulin? Is it completely altered or destroyed, or is it merely rendered inert by the addition of the trypsin molecule to it?

We have attempted to answer these questions in the following way. We drew attention above, to the fact that the precipitate which forms upon the addition of sufficient trypsin to the insulin contains the original insulin, and that this precipitate is soluble both by the addition of alkali (raising the pH of the surrounding medium), and by the addition of acid (lowering the pH). When such solutions (acid and alkali) of the precipitate are injected into rabbits (see protocols 3, 4 and 5), we find that the alkaline solution is inert, and that the acid solution produces the same insulin effects in rabbits as the control animals which received insulin alone. It appears from these results that at a proper pH (above 4.6) the trypsin attaches

itself to the insulin rendering the latter inert. Complete inactivation of insulin occurs at a pH of 4.6 to 4.8 and remains permanent as long as the pH is above 4.8 This fact is particularly noteworthy because the lowest recorded pH at which tryptic digestion can take place is between 5.5 and 6.3 (6). On the other hand, if the pH is lowered, dissociation of the insulin from trypsin takes place, and the physiological effects of the original insulin are produced in animals.

Inactivation and recovery of the insulin in the presence of trypsin may be produced at will by raising or lowering the pH above and below a certain point. This can be done repeatedly as long as the trypsin remains active. Prolonged exposure of the trypsin to a low pH ultimately destroys the trypsin and then further inactivation of the insulin by raising the pH no longer takes place.

That this process of inactivation by trypsin under the conditions described above is not the result merely of change (elevation) of the pH per se, is shown by the following experiments (see protocols 3, 4 and 6). We find in these experiments that elevation of the pH of the insulin solution even to the point of alkalinity does not in any way alter the action of the insulin.

It seems possible from the evidence presented that trypsin combines with the insulin forming an inactive product.[2] The combination is permanent under a certain set of conditions, and is dissociated under others.

It is noteworthy in this connection, that whereas insulin becomes promptly inactivated by trypsin, the latter suffers no change, either in respect to its proteolytic or rennet action, from the contact (see protocol 7).

That the inactivation of insulin by trypsin is due to the active ferment itself and not to any other agent that may be associated with it, is evidenced by the fact that such inactivaton does not take place with heated inactive trypsin (protocol 6). This point is in keeping with the observations of Witzemann and Livshis (7).

From the evidence thus far presented, the reaction between trypsin and insulin resembles that which occurs between trypsin and safranin (8). When a solution of safranin is added to trypsin, a precipitate develops. This precipitate is a combination of trypsin and safranin and possesses proteolytic properties; and, as Marston has pointed out, dissociation of the safranin from the trypsin takes place when the precipitate is dissolved in an acid solution (0.2 per cent HCl). This property of combining with trypsin Marston ascribes to the azine and azonium radicals in the safranin. The similarity of the reaction between safranin and trypsin, and that between insulin and trypsin is very suggestive and may throw some light on the structural character of insulin.

[2] For purposes of convenience, we have named the inactive product, "trypsulin."

The inactivation of insulin by trypsin appears moreover to be of a quantitative nature. Of the preparation of trypsin which was employed approximately 0.003 mgm. of the ferment inactivated 1 standard unit of insulin. The quantitative relation between the trypsin and insulin is quite definite.

That the reaction in question is not of a proteolytic character is further evidenced by the fact that serum does not hinder the action of trypsin on insulin (protocol 8). In such experiments we observed that the injection of mixtures of serum and insulin produce the physiological effects characteristic of insulin—the hypoglycemia and convulsions. On the other hand mixtures of serum and insulin, to which trypsin is added, fail to produce insulin reactions in animals. The conclusion seems justified that the inactivation of insulin by trypsin takes place in the presence of serum as well as in its absence. This would not be the case if the action of trypsin on insulin were one of proteolysis. We called attention to the fact that purified trypsin possessed a rennet action and therefore suggested that the inactivation of insulin might be due to this phase of the tryptic activity. This, however, seems unlikely from the evidence presented in protocol 7, from which it was made evident that the product (trypsulin) which formed upon the addition of trypsin to insulin retains both the proteolytic and rennet action.

Granting that physiologic inactivation of insulin by trypsin may be immediate, that it may take place at any of the temperatures stated, (3°, 18°and 37.5°C.), and that it may occur even in the presence of serum, the question might still be raised as to the ultimate fate of the insulin when trypsin is permitted to act upon it over a long period of time. Does it finally undergo proteolytic cleavage? Witzemann and Livshis (9), for example, observed that after digestion of insulin for 48 hours, the amino-acids in the solution increased in amount. If we should accept the figures which they present as dependable evidence of tryptic digestion (and they were not working with a pure trypsin) their result might be due to cleavage of protein-like impurities in the insulin, or of protein present in their preparation of pancreatin. Our answer to this question is furnished by the observation that reactivation of the insulin is still possible by proper acidification of the digest after variable periods of time (protocol 7). We find that even after a period of 42 hours' digestion the insulin can still be recovered. It would appear then that insulin is virtually indestructible and is merely rendered physiologically inert by trypsin.

The most striking demonstration of the nature of the reaction of trypsin on insulin, and the strongest argument against the theory of proteolysis, is furnished by the experiments in which the reaction was tested in vivo (protocol 9).

Here again we find that inactivation of the insulin occurs. The reaction therefore takes place not only in the test tube but in the body of the ani-

mal as well. Larger amounts of trypsin are naturally needed to produce this effect, than in vitro experiments, but that is as it should be, if we take into consideration all the conditions which surround such an experiment. This inactivation in vivo is however, subject to special conditions. When the injection of the two substances is made in close chronological order with the trypsin preceding the insulin, the inactivation takes place. When, however, the injections are made in the reverse order, or at long intervals, the inactivation is incomplete, manifesting itself by a delay in the exhibition of the physiologic reactions of insulin.

The inactivation of insulin by trypsin in vivo is not due to any neutralizing hyperglycemia incidental to the administration of trypsin because trypsin alone does not produce any significant rise in the blood sugar (protocol 10).

The results attained therefore, must be due to a direct action of the trypsin on insulin, rendering it inert, and thus checking its physiological effects on animals.

SUMMARY

To summarize, our observations show:

1. That trypsin inactivates insulin.

2. That this inactivation takes place amost instantaneously, at 3°, 18° and 37.5°C.

3. This inactivation takes place at any pH to the alkaline side of 4.6.

4. The reaction appears to be of a quantitative nature, and we have estimated that 0.003 mgm. of purified trypsin inactivates 1 standard unit of insulin.

5. Liberation or dissociation of the insulin from the trypsin can be accomplished by proper acidification (pH below 4.6).

6. Prolonged contact of trypsin with insulin does not destroy the insulin. Reactivation is still possible after 42 hours' contact at 37.5°C.

7. The action of trypsin on insulin is not of a proteolytic character; it is in the nature of a chemical reaction comparable to that which takes place between trypsin and safranin.

8. The product resulting from the combination of trypsin and insulin retains its proteolytic and milk-coagulating actions.

9. Blood serum does not hinder the effect of trypsin on insulin.

10. The inactivation of insulin by trypsin can occur within the body of an animal under suitable experimental conditions.

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PROTOCOLS

Protocol 1. 1/10/24. To 100 units of insulin (U 20, lot 73865–741139) were added 2 cc. of trypsin. The mixture was centrifuged for 5 minutes.

Experiment 1. 3.5 cc. of the supernatant fluid (representing 50 units of the original insulin) were injected into a fasted rabbit and the effect on the blood sugar was observed.

TIME	BLOOD SUGAR	REMARKS
	per cent	
11:15 a.m.	0.127	Before injection
11:25		Solution injected
12:15	0.140	
1:15	0.145	
3:15	0.141	No reaction

Experiment 2. To 3.5 cc. of the supernatant fluid 20 units (U 10) fresh insulin were added, and the mixture injected into a fasted rabbit, and the effect on the blood sugar observed.

TIME	BLOOD SUGAR	REMARKS
	per cent	
11:20 a.m.	0.132	Before injection
11:30		Solution injected
12:30	0.123	
1:30	0.125	
3:30	0.128	No reaction

Note: The blood sugar determinations were made by the Epstein modification of the Lewis-Benedict method.

Protocol 2. *Experiment 1.* To 50 units of insulin (lot 73865–741139) were added 2 cc. of trypsin. The mixture was centrifuged to separate the precipitate. The supernatant fluid was decanted off. The precipitate was then washed in several changes of distilled water. It was finally suspended in 2 cc. of distilled water and a minute amount of 10 per cent Na_2CO_3 was added to cause solution of the precipitate. The resulting solution was water clear. This solution was then injected into a fasted rabbit (weight 1.5 kgm.).

No reaction took place after 6 hours of observation.

Experiment 2. For purposes of immunization tests, 2 rabbits (weight 1.8 + kgm.) were given 12 successive injections intraperitoneally every fourth day, with alkaline solutions of the precipitate obtained by the addition of trypsin to 50 units of insulin at each injection, without producing any insulin effects.

PROTOCOL 3. 2/21/24. Testing the effect of precipitation and solution of insulin by means of alkali with and without the addition of trypsin on the activity of insulin.

Experiment 1. To 1 cc. of insulin (U 20, lot number 74879–741147) was added 0.1 cc. trypsin and 0.03 cc. of a 10 per cent Na_2CO_3 solution. The mixture was injected into a fasted rabbit (weight 1.0 kgm.) at 2 p.m.

Result: No reaction.

Experiment 2. To 1 cc. of insulin (U 20 same lot number as above) was added n/10 NaOH in amount sufficient to cause precipitation of the insulin and then dissolve it. This alkaline insulin was injected into a fasted rabbit (weight 1.2 kgm.) at 2 p.m.

Result: Violent convulsions at 3:45 p.m., i.e., $1\frac{3}{4}$ hours later.

Experiment 3. To 1 cc. of insulin (U 20 same as above) was added a buffered solution pH 7.8 in amount sufficient to cause precipitation of the insulin and then to dissolve it. This alkaline insulin was injected into a fasted rabbit (weight 1.4 kgm.) at 2:05 p.m.

Result: Violent convulsions at 3:25 p.m., i.e., 1 hour and 20 minutes later.

Experiment 4. 1 cc. of insulin (U 20, same lot number as above) was injected into a fasted rabbit (weight 1 kgm.) at 2 p.m. (Control.)

Result: Violent convulsions at 3 p.m., i.e., 1 hour later.

Deduction: Alkalinization of the insulin does not affect its activity, whereas the addition of trypsin renders it immediately inactive.

PROTOCOL 4. 1/10/24. Testing for the *reactivation* of insulin first rendered inactive by trypsin.

To 100 units of insulin (U 20, lot 73848–741135) 2 cc. trypsin were added. The precipitate which formed was separated by centrifugalization and washed in several changes of water (distilled) to remove excess of trypsin. The precipitate was then suspended in 5 cc. of water and enough 10 per cent Na_2CO_3 solution added to cause complete solution. One portion of this alkaline solution was divided into 2 doses and injected into rabbits as controls (see expts. 1 and 2). The balance was acidified and portions representing 10, 15 and 20 units of insulin were injected into rabbits (see expts. 3, 4 and 5).

Experiment 1. 0.85 cc. representing 15 units of the original insulin was injected into a fasted rabbit at 11:30 a.m.

Result: No reaction.

Experiment 2. 1.1 cc. representing 20 units of the original insulin were injected into a fasted rabbit at 11:30 a.m.

Result: No reaction.

Experiment 3. 0.60 cc. representing 10 units of insulin was injected into a fasted rabbit at 12 noon.

Result: Convulsions developed at 2:10 p.m.; glucose was given subcutaneously and convulsions were checked.

Experiment 4. 1.1 cc. representing 15 units of insulin were injected into a fasted rabbit.

TIME	BLOOD SUGAR	REMARKS
	per cent	
11:40 a.m.	0.125	Before injection
12:05 p.m.		Solutions injected
12:35	0.096	
1:05	0.074	
2:20		Convulsions, glucose given subcutaneously

Experiment 5. 1.4 cc. representing 20 units of insulin was injected into a fasted rabbit at 12:10 p.m.

Result: Convulsions developed at 1:45 p.m.; glucose given and convulsions checked.

Experiment 6. 0.75 cc. fresh insulin (U 20, lot number same as above) representing 15 units was injected into a fasted rabbit at 12:15 p.m. (Control.)

Result: Convulsions developed at 2:12 p.m.; glucose was injected and the convulsions checked.

Deduction: It is obvious from the experiments in this protocol as well as in the preceding one that trypsin inactivates insulin by combining with it. This product remains inactive in alkaline solutions. On the other hand, acidification causes reactivation or dissociation of the insulin.

PROTOCOL 5. 3/4/24. Testing the effect of prolonged contact (digesting in thermostat at 37.5°C.) between trypsin and insulin, on the fate of the latter, and the possibility of its reactivation.

Experiment 1. To 1 cc. of insulin (U 40, lot 75142-741148) was added 0.2 cc. trypsin solution and enough n/10 NaOH to cause solution of the precipitate which formed.

a. One portion representing 25 units of the original insulin was injected immediately into a fasted rabbit (weight 1.4 kgm.). (Control.)

Result: No reaction after 6 hours observation.

b. The remaining portion representing 15 units of the original insulin was acidified with HCl and injected immediately into a fasted rabbit (weight 1.5 kgm.).

Result: Convulsions 1 hour and 10 minutes later, glucose injected and convulsions controlled.

Experiment 2. To 1 cc. of insulin (U 40, same lot number as above) was added 0.2 cc. trypsin solution and enough n/10 NaOH to cause solution of the precipitate which formed. Incubated in thermostat at 37.5°C. for 30 minutes.

a. One portion representing 25 units of the original insulin was injected into a fasted rabbit (weight 1.2 kgm.). (Control.)

Result: No reaction after 6 hours' observation.

b. The remaining portion representing 15 units of the original insulin was acidified with HCl and injected into a fasted rabbit (weight 1.5 kgm.).

Result: Convulsions developed 1 hour and 10 minutes later. Glucose injection controlled the convulsions.

Experiment 3. To 1 cc. of insulin (U 40, same lot number as above) was added 0.2 cc. trypsin solution and enough n/10 NaOH to cause solution of the precipitate which formed. Incubated in thermostat at 37.5°C. for 1 hour.

a. One portion representing 25 units of the original insulin was injected into a fasted rabbit (weight 1.4 kgm.). (Control.)

Result: No reaction after 6 hours' observation.

b. The remaining portion was acidified with HCl and injected into a fasted rabbit (weight 1.3 kgm.)

Results: Convulsions developed 1 hour and 45 minutes later. Glucose controlled the convulsions temporarily; recurrence of convulsions 30 minutes later which were finally controlled by additional injections of glucose.

Experiment 4. To 1 cc. of insulin (U 40, same lot number as above) was added 0.2 cc. trypsin solution and enough n/10 NaOH to cause solution of the precipitate which formed. Incubated in thermostat at 37.5°C. for 24 hours.

a. One portion representing 20 units of the original insulin was injected into a fasted rabbit (weight 1.5 kgm.). (Control.)

Result: No reaction after 6 hours' observation.

b. The remaining portion representing 20 units of the original insulin was acidified and injected into a fasted rabbit (weight 1.5 + kgm.).

Result: Convulsions developed 2 hours later. Injections of glucose controlled the convulsions.

Experiment 5. Additional control test. 0.5 cc. of insulin (U 40, same lot number as above) was injected into a fasted rabbit (weight 1.2 kgm.).

Result: Convulsions 1 hour and a half later, controlled by injections of glucose.

Deduction: Reactivation of insulin is possible even after 24 hours' contact with trypsin under conditions favorable for digestion.

In another set of experiments we found that reactivation of insulin is possible even after 42 hours' digestion by trypsin.

Note: The trypsin used in the above experiments was of high potency: 0.00025 cc. digested 2 cc. of casein in ½ *hour*. (Gross-Fuld method.)

PROTOCOL 6. 2/23/24. In these experiments an attempt is made to ascertain the following points:

1. The difference in the effect of active trypsin and boiled or inactivated trypsin on insulin.

2. The effect of raising the pH or alkalinization of insulin on its activity.

Experiment 1. To 1 cc. of insulin (U 20, lot 74879–741147) was added 0.2 cc. of the trypsin solution which had been previously boiled for 5 minutes, and enough n/10 NaOH to dissolve the precipitate which formed on the addition of the trypsin. This mixture after 5 minutes' contact was injected into a fasted rabbit (wt. 1.4 kgm.).

Result: Convulsions in 1 hour and 15 minutes; glucose injected and convulsions controlled.

Experiment 2. To 1 cc. of insulin (U 20, same lot number as above) was added only 0.1 cc. of active trypsin solution, and enough n/10 NaOH to dissolve the precipitate which formed. The mixture after 5 minutes' contact was injected into a rabbit (wt. 1.2 kgm.).

Result: No reaction after 6 hours' observation.

Experiment 3. To 3 cc. of insulin (U 20, same lot number as above) was added powdered $CaCO_3$ (c.p.); the mixture was shaken long enough to assure the solution of some calcium. The solution was then filtered.

a. 1 cc. was used to determine the pH, which was found to be 7.6, and

b. 1 cc. was injected into a fasted rabbit (wt. 1.5 kgm.).

Result: Convulsions 1 hour and 20 minutes later; convulsions checked by injections of glucose.

Experiment 4. To 1 cc. of insulin (U 20, lot number same as above) was added enough of a buffered solution of pH 7.8 to cause precipitation and solution of the insulin. The mixture after 5 minutes' contact was injected into a fasted rabbit (wt. 1.5 kgm.).

Result: Convulsions developed in 1 hour and 15 minutes. Glucose injected and convulsions ceased.

Deduction: 1. That active trypsin inactivates insulin in an alkaline medium after 5 minutes' contact.

2. That boiled (inactivated) trypsin does *not* inactivate insulin under like conditions.

3. That raising the pH, or alkalinizing the insulin does not impair the activity of the insulin.

4. That the presence of calcium does not enter as a factor in the process of inactivation of the insulin by trypsin. The reason for this test is that in our trypsin solution some calcium is present.

PROTOCOL 7. The object of this experiment was to ascertain the effect of insulin on the trypsin.

Experiment 1. To 5 cc. or 100 units of insulin was added enough trypsin solution to cause complete precipitation. The mixture was centrifuged and the supernatant fluid removed by decantation. The precipitate was washed in four changes of distilled water to remove the excess of trypsin. It was centrifuged after each washing. Finally the precipitate was suspended in 2 cc. of distilled water and enough n/10 NaOH was added to dissolve it.

a. One-half of the solution was added to casein and tested for tryptic activity (Gross–Fuld method). Complete digestion occurred in 1½ hours.

b. The other half was added to 5 cc. of milk to which a drop of $CaCl_2$ solution was added. Coagulation of the milk occurred in 1 hour.

Deduction: Trypsin combines with insulin. The combination which forms, though inactive for insulin (see preceding protocols), is active tryptically, and can coagulate milk. The calculated amount of trypsin which has been bound to the insulin is 0.3 mgm.

PROTOCOL 8. 1/5/24. The object of these experiments was to ascertain the effect of blood serum on the action of trypsin on insulin.

Experiment 1. 10 units of insulin (lot 73848–741135) were injected into a fasted rabbit (wt. 1.6 kgm.). Control.

TIME	BLOOD SUGAR	REMARKS
	per cent	
10:15 a.m.	0.135	Before injection
10:20		Insulin injected
11:20	0.090	
12:20	0.065	
12:35		Violent convulsions, glucose given

Experiment 2. 20 units of insulin (lot number same as above) were injected into a fasted rabbit (wt. 1.85 kgm.) at 10:05 a.m. Control.

Result: Convulsions developed at 11:40 a.m. Glucose was given and convulsions checked.

Experiment 3. To 10 units of insulin (lot number same as above) was added 1 cc. of blood serum. After 5 minutes' contact the mixture was injected into a fasted rabbit (wt. 1.6 kgm.). Serum+Insulin Control.

TIME	BLOOD SUGAR	REMARKS
	per cent	
10:00 a.m.	0.138	Before injection
10:10		Injection of serum and insulin
11:15	0.080	
12:15		Convulsions, glucose given

Experiment 4. To 20 units of insulin (lot number same as above) was added 1 cc. blood serum. After 5 minutes' contact the mixture was injected into a fasted rabbit (wt. 1.9 kgm.). Serum+Insulin Control at 10:12 a.m.

Result: Convulsions developed at 11:45 a.m.

Experiment 5. To 10 units of insulin (lot number same as above) was added 1 cc. of blood serum and 0.25 cc. trypsin. After 5 minutes' contact the mixture was injected into a fasted rabbit (wt. 1.6 kgm.) at 9:40 a.m.

Result: No reaction after 6 hours' observation.

Experiment 6. To 20 units of insulin (lot number same as above) was added 1 cc. of blood serum and 0.25 cc. trypsin. After 5 minutes' contact the mixture was injected into a fasted rabbit (wt. 1.8 kgm.).

TIME	BLOOD SUGAR	REMARKS
	per cent	
9:50 a.m.	0.141	Before injection
10:00		Injection of the mixture of insulin + serum + trypsin
11:00	0.121	
12:00 noon	0.122	
		No convulsions after 6 hours' observation

Deduction: Serum added to insulin does not impair its activity. The amount of serum used is many times that required for the antitryptic action of the trypsin employed.

PROTOCOL 9. 12/19/23. Testing the effect of trypsin on insulin in vivo.

Experiment 1. Fasted rabbit (wt. 1.6 kgm.) was given 10 units insulin intravenously (ear vein) at 1:30 p.m. Insulin control.

Result: Convulsions developed at 3 p.m., glucose injections relieved the convulsions.

Experiment 2. Fasted rabbit (wt. 1.8 kgm.) was given 5 cc. of trypsin solution intravenously (ear vein) at 1:35 p.m. Trypsin control.

Result: No reaction.

Experiment 3. Fasted rabbit (wt. 1.65 kgm.) was given 11 units of insulin intravenously (ear vein), and immediately thereafter 5 cc. trypsin solution intravenously into the other ear vein, at 1:45 p.m.

Result: No reaction.

Experiment 4. Fasted rabbit (wt. 1.8 kgm.) was given 14 units of insulin intravenously in one ear vein, and immediately thereafter 5 cc. of trypsin solution intravenously into the other ear vein, at 2 p.m.

Result: No reaction.

Experiment 5. Fasted rabbit (wt. 1.15 kgm.) given 5 cc. trypsin solution subcutaneously at 10:30 a.m., and 15 units of insulin subcutaneously 5 minutes later.

Result: Convulsions at 12:00 noon; checked by injections of glucose.

Experiment 6. Fasted rabbit (wt. 1.5 kgm.) given 5 cc. trypsin solution subcutaneously at 10:35 a.m., and 15 units of insulin 30 minutes later.

Result: Convulsions at 3:05 p.m. which were checked by glucose injections.

Experiment 7. Fasted rabbit (wt. 1.6 kgm.) given 5 cc. trypsin solution subcutaneously at 10:38 a.m. amd 15 units of insulin 1 hour later.

Result: No convulsions.

Experiment 8. Fasted rabbit (wt. 1.1 kgm.) was given 5 cc. trypsin solution subcutaneously at 10:38 a.m. and 15 units of insulin 2 hours later.

Result: Convulsions at 2 p.m., checked by glucose injections.

Deduction: Trypsin checks insulin action when the two agents are given simultaneously by the intravenous route. When given subcutaneously the result is uncertain; occasionally checking of the insulin action occurs, provided the trypsin is given first. At other times only a delay in the insulin action is obtained.

PROTOCOL. 10. 2/5/24. The object of these experiments was to test the effect of trypsin on the blood sugar.

Experiment 1. Rabbit (wt. 1.0 kgm.) fasted for 24 hours.

TIME	BLOOD SUGAR	REMARKS
	per cent	
12:10 p.m.	0.140	
12:15		0.5 cc. trypsin solution intravenously
1:15	0.141	
2:15	0.140	
3:45	0.130	

Experiment 2. Rabbit (wt. 1.0 + kgm.) fasted for 24 hours.

TIME	BLOOD SUGAR	REMARKS
	per cent	
12:00 noon	0.123	
12:05 p.m.		0.75 cc. trypsin solution intravenously
1:10	0.141	
2:10	0.121	
3:40	0.141	
4:15	0.135	

Deduction: Trypsin does not affect the blood sugar content. The trypsin was given intravenously rather than subcutaneously so as to obviate any question as to the rate of absorption.

SOME CHEMICAL CHANGES OF THE BLOOD PRODUCED BY DRUGS

III. Phlorhizin

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This study was undertaken to obtain a more complete picture of the changes which take place in the metabolism of a phlorhizinized animal, as shown by blood analysis, and perhaps in this way throw new light upon the mechanism of phlorhizin diabetes.

Phlorhizin causes a glucosuria and a consequent hypoglycemia, as has been shown by Von Mering (1), Zuntz (2), Underhill (3), Nash (4) and others There is also evidence now to show that the completely phlorhizinized animal cannot utilize sugar, as shown by the quantitative recovery of ingested glucose in the urine. Ringer (5), Nash and Benedict (6) and Lusk (7) have shown that the respiratory quotient is not changed by sugar ingestion in such animals. That this inability to utilize sugar is not due to the hypoglycemia has been demonstrated by Nash and Benedict (6), who gave enough glucose to produce a hyperglycemia in a completely phlorhizinized dog and still obtained a complete recovery of the ingested sugar in the urine.

Other actions of phlorhizin have been recorded in the literature. Levene (8) found that the bile contained some reducing substance after the administration of phlorhizin, and his results have been confirmed by Woodyatt (9). Pearce (10) has shown that phlorhizin caused the appearance of reducing substances in the digestive juices.

Methods. The dogs used in this investigation were kept on a meat diet throughout the entire time of the experiment. They were fasted for twenty hours before taking blood samples, so that there would be no effect of digestion on the composition of the blood analyzed. The sample of blood was drawn from the left ventricle and was of such a size that any changes found could not be due to the effect of hemorrhage. The normal composition of the blood was obtained from samples taken from the dog on several different days. Phlorhizin was then given subcutaneously in an alcoholic solution, each animal receiving 2 grams daily, 1 gram early in the morning,

and the other late in the afternoon. Three to four hours elapsed between the injection of the drug and the drawing of the blood sample. The administration of the drug was usually continued for three or four days so as to be certain that all changes would be observed.

One animal was used to make sure that the amount and method of phlorhizinization was comparable to methods used by other investigators. This dog was placed in a metabolism cage and starved for the length of the experiment. The urine was collected and analyzed for sugar and nitrogen so that the D:N ratio might be calculated. In other ways the procedure was the same as with the other seven dogs.

The methods used in the blood analysis were the same as those discussed elsewhere (11). The carbon dioxide combining capacity of the blood was determined by the method of Van Slyke (12); the sugar, non-protein nitrogen and creatinine by the methods of Folin and Wu (13); the urea nitrogen by the method of Van Slyke and Cullen (14); total fat (15) and cholesterol (16) according to Bloor. The hydrogen ion concentration was determined by the potentiometric method, using the Clark electrode and the saturated calomel cell. Through the use of these micro methods all the determinations were made on one sample of blood. The urinary sugar was determined by the method of Benedict (17), and urinary nitrogen by micro-Kjeldahl procedure (18). All analyses were run in duplicate.

EXPERIMENTAL DATA. All the dogs showed the typical effects of the drug; sugar was found to be present in the urine, and there was a gradual depression and loss of appetite as the poisoning was continued.

The results of the blood analysis, obtained with eight dogs, are given in table 1. An average was made of the normal analyses and another of the analyses after the injection of the drug, and from these averages the percentage of change was calculated.

It will be observed that there was always a decrease in the carbon dioxide combining capacity, the average change being 24 per cent. The sugar also shows a constant lowering with an average difference of 29 per cent. The nitrogen constituents show a persistent increase in value, the mean increase for non-protein nitrogen being 24 per cent, for urea nitrogen 44 per cent, for creatinine nitrogen 21 per cent. The cholesterol in general shows a large increase, although two animals show small decrease. The total fat also shows a consistent increase after phlorhizin with an average change of 29 per cent. The hydrogen ion concentration shows a decrease in every animal.

The results of the metabolic experiment in the blood analysis show that the reaction was the same as with the other animals. The D:N ratios of 3.6 and 3.48 obtained, also indicate that the dog was completely phlorhizinized.

DISCUSSION OF RESULTS. The results obtained in these experiments are, in general, confirmatory of those previously obtained by other methods.

TABLE 1

Dog 10, weight 13.6 kilos

DATE	CO_2	SUGAR	N.P.N.	CREATININE	CHOLESTEROL	pH	REMARKS
	vol. per cent	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*		
2/23	49.0	0.086	21.2	1.34			Normal
2/24	52.0			1.29	0.131		Normal
2/27	49.0	0.089	22.1	1.30	0.128		Normal
2/28	49.0	0.098	22.2	1.29	0.131	7.34	Normal
	49.7	0.088	21.8	1.30	0.130	7.34	Normal average
3/1	45.0	0.061	25.1	1.52	0.125	6.99	Phlorhizin
3/2	32.0	0.072	25.6	1.50	0.121	7.01	Phlorhizin
3/3	30.0	0.071	25.6	1.32	0.113	7.02	Phlorhizin
	35.6	0.068	25.4	1.44	0.120	7.01	Average after drug
	−28.4	−22.6	+16.5	+10.7	−7.7	−4.5	Per cent change

Dog 4, weight 12.4 kilos

DATE	CO_2	SUGAR	N.P.N.	CREATININE	UREA	CHOLESTEROL	REMARKS
	vol. per cent	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	
3/15	46.0	0.091	28.2			0.167	Normal
3/16	46.0	0.111	28.1	1.67		0.167	Normal
3/17	48.0	0.091	26.6	1.95	10.3	0.167	Normal
	46.6	0.097	27.6	1.81	10.3	0.167	Normal average
3/20	43.0	0.081	33.6	1.95	10.1	0.510	Phlorhizin
3/21	39.0	0.081	30.8	1.95		0.575	Phlorhizin
3/22	46.0	0.070	30.1	1.84		0.592	Phlorhizin
3/23	39.0	0.063	30.8	1.96	33.6	0.609	Phlorhizin
3/24	37.0	0.063	28.0	1.62	22.4	0.400	Phlorhizin
	40.8	0.072	30.7	1.86	22.0	0.537	Average after drug
	−17.41	−35.0	+11.2	+2.76	+113.0	+222.0	Per cent change

Dog 18, weight 10 kilos

DATE	CO_2	SUGAR	N.P.N.	CREATININE	UREA	CHOLESTEROL	TOTAL FAT	pH	REMARKS
	vol. per cent	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	*per cent*		
10/6	45.0	0.097							Normal
10/10	50.0	0.115	23.8	1.01	17.7	0.166	0.73		Normal
10/16	49.5	0.109	21.0	1.10	12.1	0.156	0.93	7.34	Normal
	48.1	0.107	22.4	1.06	14.9	0.161	0.83	7.34	Normal average

TABLE 1—*Continued*

Dog 18, weight 10 kilos—Continued

DATE	CO_2	SUGAR	N.P.N.	CREATININE	UREA	CHOLESTEROL	TOTAL FAT	pH	REMARKS
	vol. per cent	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	*per cent*		
10/18	34.0	0.072	32.2	1.20	36.4	0.152	0.85	7.36	Phlorhizin
10/19	30.0	0.061	27.8	1.12		0.164	1.02		Phlorhizin
10/20	28.0	0.091	60.1	2.50		0.156	1.04	7.24	Phlorhizin
	30.6	0.074	40.0	1.61	36.4	0.157	0.97	7.30	Average after drug
	−36.3	−30.8	+78.5	+51.9	+144.0	−2.49	+16.9	−5.45	Per cent change
				Dog 19, weight 9.6 kilos					
10/7	44.5	0.094			16.25				Normal
10/10	46.5	0.113	28.0	1.15	19.6	0.132	0.940	7.44	Normal
10/11	46.0	0.115	22.4				0.80	7.39	Normal
10/16	47.1	0.107	29.4	1.23	19.6	0.156	0.960	7.44	Normal
	48.0	0.107	26.6	1.19	18.48	0.144	0.900	7.42	Normal average
10/18	37.0	0.104	39.2	1.41	33.6	0.164	0.89		Phlorhizin
10/19	41.0	0.111	32.2	1.25		0.173	0.96	7.30	Phlorhizin
10/20	36.5	0.065	35.0	1.12		0.143	0.89	7.26	Phlorhizin
	38.1	0.093	35.4	1.26	33.6	0.160	0.910	7.28	Average after drug
	−20.6	−13.1	+33.1	+5.89	+76.4	+11.1	+1.1	−18.8	Per cent change
				Dog 20, weight 13.6 kilos					
12/4	53.0	0.108	25.2	0.95		0.129		7.44	Normal
12/5	50.0	0.099	32.2	1.15	16.54	0.156	0.75	7.34	Normal
12/8	47.0	0.122	25.6	1.43	18.6	0.147	0.69	7.33	Normal
12/11	51.0	0.090	28.1		21.2	0.156	0.96	7.50	Normal
	50.2	0.106	27.7	1.14	18.78	0.147	0.80	7.40	Normal average
12/13	44.0	0.091	23.8			0.151	0.88	7.25	Phlorhizin
12/14	35.0	0.071	39.8		20.5	0.169	0.68	7.25	Phlorhizin
12/15	33.0	0.071	30.8		19.6	0.178	0.84	7.37	Phlorhizin
	37.3	0.078	31.2		20.0	0.166	0.80	7.29	Average after drug
	−25.8	−26.4	+12.6		+6.5	+12.8	0	−14.9	Per cent change
				Dog 21, weight 7.5 kilos					
12/4	51.0	0.114	27.3			0.156	0.86	7.38	Normal
12/5	49.0	0.097	28.0	1.05	11.2	0.156	0.83	7.25	Normal
12/8	48.0	0.127	26.6	1.36	18.6	0.183	0.94	7.44	Normal
12/11	41.0	0.097	30.5		17.7	0.156	0.84	7.21	Normal
	47.2	0.108	28.1	1.20	15.8	0.162	0.86	7.32	Normal average

TABLE 1—*Concluded*

Dog 21, weight 7.5 kilos—Continued

DATE	CO_2	SUGAR	N.P.N.	CREATININE	UREA	CHOLESTEROL	TOTAL FAT	pH	REMARKS
	vol. per cent	*per cent*	*mg. per 100 cc.*	*mg. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	*per cent*		
12/13	41.0	0.086	32.2			0.178	0.88	7.34	Phlorhizin
12/14	35.0	0.056	39.2	1.59	23.6	0.192	1.02	7.25	Phlorhizin
12/15	33.0	0.064	23.9		14.0	0.204	1.83	7.31	Phlorhizin
	36.3	0.068	31.7	1.59	18.8	0.191	1.24	7.30	Average after drug
	−23.1	−37.1	+12.8	+32.5	+19.0	+17.9	+44.1	−2.72	Per cent change

Dog 22, weight 14.9 kilos

DATE	CO_2	SUGAR	N. P. N.	CREATININE	UREA	CHOLESTEROL	TOTAL FAT	pH	REMARKS
	vol. per cent	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	*per cent*		
1/16	42.1	0.120	26.6	1.76	28.9	0.156	0.89	7.36	Normal
1/17	50.0	0.099	32.2	2.00		0.151	0.80		Normal
2/1	45.5	0.097		1.39	13.1	0.139	1.03	7.38	Normal
2/2	43.0	0.089	26.6	1.39	18.6	0.113	0.81	7.34	Normal
	45.1	0.101	28.4	1.63	15.8	0.139	0.88	7.36	Normal average
2/6	31.0	0.059	28.0	1.34	12.2	0.180	1.44	7.29	Phlorhizin
2/7	42.0	0.065	29.4	2.62	21.4	0.180	1.83	7.40	Phlorhizin
	36.5	0.062	28.7	1.98	16.8	0.180	1.63	7.34	Average after drug
	−18.1	−38.6	+1.06	+21.5	+6.33	+29.6	+85.1	−2.72	Per cent change

Dog 24, weight 14.8 kilos

DATE	CO_2	SUGAR	N.P.N.	CREATININE	CHOLESTEROL	TOTAL FAT	pH	D/N	REMARKS
	vol. per cent	*per cent*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	*per cent*			
6/9	41.0	0.095	25.2	143	119	161	7.43		Normal
6/10	41.0	0.100	28.0	150	119	173	7.45		Normal
6/11	41.0	0.103	24.2	143	125	200	7.44		Normal
	41.0	0.099	25.8	145	121	178	7.44		Normal average
6/13	36.0	0.102	33.6	143	120	214	7.42	5.02	Phlorhizin
6/14	33.0	0.065	36.4	187	132	200	7.46	3.60	Phlorhizin
6/15	35.0	0.056	32.2	219	139	225	7.45	3.48	Phlorhizin
	35.0	0.072	34.0	183	130	213	7.44		Average after drug
	−14.7	−27.2	+21.2	+28.0	+7.45	+19.7	0		Per cent change

Von Mering (1) was the first to show that phlorhizin caused a glucosuria, and he also asserted that there was a hypoglycemia. His assertion was confirmed by numerous authors: Minkowski (19), Levene (8), Czyhlarz and Schlesinger (20), Jacoby and Rosenfeld (21) and others. Csonka (22) showed that the drug produced a hypoglycemia, but that if protein or carbohydrates were given a hyperglycemia resulted. Ingestion of fat produced no hyperglycemia. Sansum and Woodyatt (23) demonstrated that doses of epinephrin given with injections of phlorhizin caused a complete removal of all the sugar from the body. They point out that in the study of new sugar formation, each animal should be tested by this method to be certain that it is sugar-free.

Levene (8) in 1894 showed that there was an increase in the protein and total fat constituents of the blood, and the results of this investigation are confirmatory of the increase in total lipoids.

TABLE 2

Urine analysis

Dog 24, weight 14.8 kilos, female, last feeding 6/8/23

DATE	TIME OF COLLECTION	VOLUME	NITROGEN	SUGAR	D/N	ACETONE BODIES	REMARKS
			grams	*grams*			
6/ 9/23	9:15 a.m.	402					Normal fasting
6/10/23	9:30 a.m.	375					Normal fasting
6/11/23	9:30 a.m.	368	3.02				Normal fasting
6/12/23	—	—	—	—		—	Phlorhizin
6/13/23	9:30 a.m.	380	8.86	17.6	5.02	Positive	Phlorhizin
6/14/23	9:30 a.m.	446	12.02	28.9	3.60	Positive	Phlorhizin
6/15/23	9:30 a.m.	420	12.17	32.6	3.48	Positive	Phlorhizin

The results obtained in these experiments—an acidosis, a decrease in the blood sugar, an increase in the blood non-protein nitrogen constituent, and an increase in the lipoid constituents—indicate a pathological metabolism for carbohydrates, and an explanation of these results can best be obtained by adopting the theory of Ringer (24) that phlorhizin acts in two ways: first, to lower the renal threshold for sugar, and second, to prevent the production of the internal secretion of the pancreas. Nash and Benedict (6) have shown that phlorhizin prevents the burning of sugar in dogs, and both Nash (25) and Ringer (24) have produced evidence to show that phlorhizinized dogs are able to utilize glucose when given insulin. Allen intimates that phlorhizin causes a derangement of the transportation and storage function of carbohydrates, which prevents their utilization, but that the drug causes no fundamental defect, such as is present in true diabetes.

CONCLUSIONS

Phlorhizin produces the following changes in the blood: a hypoglycemia, an increase in the total lipoids and cholesterol, an acidosis as indicated by the fall in alkali reserve and the hydrogen ion concentration, and an increase in the non-protein nitrogen.

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STUDIES IN GASTRIC SECRETION

II. Gastric Secretion in Sleep

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These experiments were undertaken to ascertain *a*, whether or not the gastric phenomena occurring during hypnosis are present in normal sleep; and *b*, to study the relation of the fractional curve to the condition of sleep.

Literature. A review of the most pertinent literature having a bearing on gastric secretion during hypnotic sleep is included in the bibliography of a previous communication (1) by Luckhardt and Johnston. They concluded that hypnotic sleep itself resulted in temporary removal of central inhibition from the gastric secretory mechanism.

Methods. The subject of the following experiments was a healthy male about twenty-four years of age. The secretory activity of the stomach was on a par with that in the subject used for experiments on psychic secretion (1). The same technique was followed in these experiments. One cubic centimeter of gastric juice, obtained by means of a Rehfuss tube and filtered when necessary, was diluted with distilled water and titrated with N/40 NaOH solution using dimethylaminoazobenzene as indicator for free acid and phenolphthalein for total acid. Specimens were aspirated at fifteen-minute intervals, the stomach being always drained at each interval except after the administration of the test meal. During the digestion of the test meal small portions were taken for filtration to yield several cubic centimeters of filtered juice. The subject kept the small modified Rehfuss tube *in situ* throughout each experiment.

Results. *The continuous secretion of gastric juice in sleep, without food.* Figure 1 shows a composite graph of three experiments performed at night. It can be seen that preparation for sleep results in a gradual rise in gastric acidity. This rise is continuous with the advent of actual sleep, reaching a height equivalent to that attained in the digestion of a test meal, but after a period of about thirty minutes the acidity begins to fall and becomes normal in about one and one-half hours after the onset of sleep. Average volumes are also shown on the graph. It should be noted that the volumes are lower in sleep than in the waking state, also as the acidity

rises the volume falls and as the volume rises the acidity falls. In view of this fact a new method of plotting curves of gastric activity is hereby proposed. This method consists of dividing the volume by ten and multiplying the resulting figure by the actual free acidity of the specimen. The quotients thus obtained are then plotted on the same graphs and form a "potentiality curve" of gastric secretion. We propose the term "potentiality" because such a curve does not take into account the enzyme content of each specimen, but is a true index of gastric efficiency when pepsin is shown to be present in adequate amounts.

TABLE 1

TIME	VOLUME	FREE ACID	TOTAL ACID	
	cc.			
9:05 p.m.	37	0.054	0.081	Residue plus some water swallowed
9:20	2.5	0.099	0.144	
9:35	7.0	0.090	0.135	
9:50	8.0	0.081	0.126	Prepares to retire; retires 10:00 p.m.
10:05	5.4	0.099	0.144	Not yet asleep. Juice slightly viscous
10:20	4.0	0.216	0.261	Appears asleep. Juice watery
10:35	2.2	0.279	0.333	Juice watery
10:50	4.2	0.306	0.360	Juice watery
11:05	3.7	0.297	0.351	Juice watery. Sleeper turns in bed
11:25	5.0	0.153	0.207	Juice viscous. Sleeper turns in bed
11:40	3.4	0.126	0.180	Juice viscous
11:55	4.7	0.126	0.180	Juice very viscous
12:10	1.0	0.126	0.180	Viscous. Sleeper aroused and talked to about steak at 12:12
12:25	20.0	0.090	0.144	Juice watery
12:40	18.0	0.126	0.180	Somewhat watery
12:55	19.0	0.189	0.234	Somewhat watery
1:10 a.m.	4.0	0.189	0.234	Somewhat watery

*Subject ate nothing since 2:00 p.m. Juice in stomach at 9:00 p.m. very scant; this time stomach tube was swallowed with the aid of a little water. If the reader will compute the potentiality curve (volume times acidity divided by ten) in the last six specimens in this table the effect of suggestion will become very evident.

The three graphs were practically identical, and in two additional experiments the *tendency* was identical, but due to broken sleep in becoming accustomed to the stomach tube they were too irregular to be considered with those averaged in figure 1. Table 1 is typical of experiments averaged in figure 1.

In figure 2 we took the free acidities and the volumes shown in figure 1 and treated them as described above to obtain the potentiality curve, and the latter is shown as a line of circles.

Figure 3 includes a graph by Kohlschutter which indicates that sleep reaches its greatest intensity in one hour and then very rapidly dimi-

nishes in intensity during the next thirty minutes. Upon this we have superimposed those free acidities in figure 1 which were obtained during sleep, taking as the beginning of sleep the period midway between the

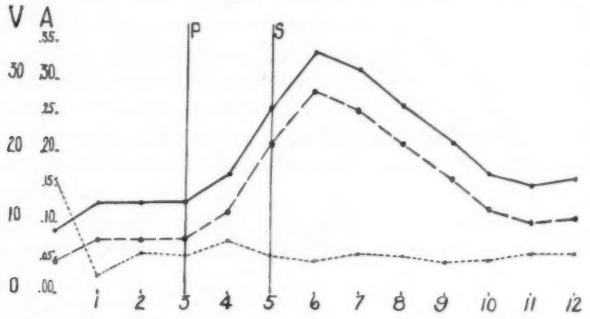

Fig. 1. Gastric secretions in sleep, without food. A composite graph of three experiments performed at night. Intervals of fifteen minutes marked below. *A* is the acidity per hundred, *V* the volume in cubic centimeters. *P* represents the time of preparation for sleep and S the time of apparent sleep. ——— Total acidity; – – – – – Free acidity; - - - - - - - - Volume of gastric juice.

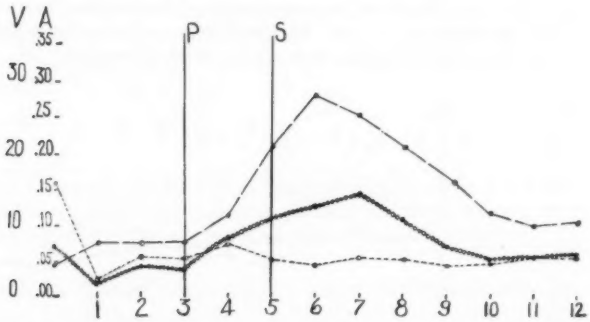

Fig. 2. Gastric secretion in sleep without food. A composite graph of the volumes and free acidities in three experiments performed at night. Intervals of fifteen minutes marked below. *A*, acidity per hundred; *V*, volume in cubic centimeters; *P*, preparation for sleep; *S*, apparent sleep; – – – – – Free acidity, - - - - - - - - volume curve of gastric juice; o o o o o potentiality curve (v × A/10).

preparation for sleep and the appearance of sound sleep, or quarter hour period *4* in figure 1. From this we conclude that the rise in gastric acidity occurring at the onset of sleep is in general proportional to the intensity of sleep, and likewise, that the acidity rapidly diminishes as the intensity of sleep diminishes.

Gastric acidity in sleep after the Ewald test meal. Figure 4 represents the free acidities of three experiments in one graph, subject in bed. In one

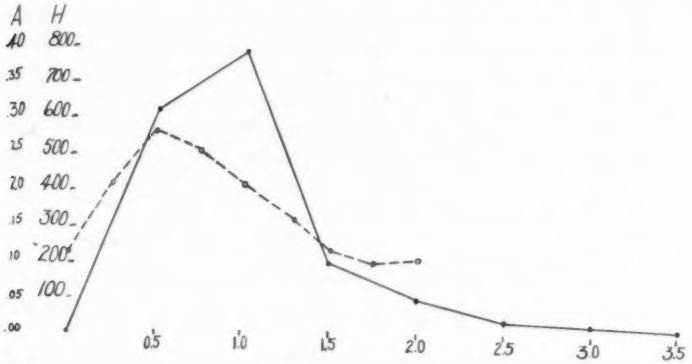

Fig. 3. Curve illustrating the strength of an auditory stimulus (a ball dropping from a height) necessary to awaken a sleeping person. Hours marked below. The tests were made at half-hour intervals. The curve indicates that the distance through which it was necessary to drop the ball increased during the first hour, and then diminished, at first rapidly, then slowly (Kohlschutter). From Howell's *Textbook of Physiology*. Upon this graph we have superimposed the graph of free acidities of figure 1 obtained during sleep. ———— degree of sleep; - - - - - free acidity during sleep: *A*, per cent of acidity; *H* height from which ball was dropped.

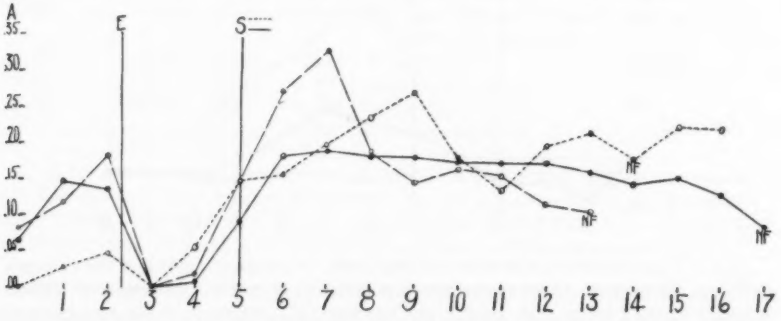

Fig. 4. Curves representing the free acidities of three experiments, subject in bed. Intervals of fifteen minutes marked below. - - - - - free acidity, subject awake. - - - - - - - - and ———— free acidities, subject asleep. *A*, percentage acidity. *E*, Ewald test meal (modified), consisting of two Uneeda biscuits and 200 cc. of water. *S*, apparent sleep. *NF*, no food in stomach.

of these the subject was unable to sleep. From this graph it can be seen that—

a. The acidity in sleep with the Ewald test meal remains at a high level very much longer than when the subject is awake. (Compare with fig. 5 showing graph with Ewald meal in the waking state.)

b. The emptying time of the stomach, following the Ewald meal, is nearly doubled in sleep what it is when the subject is walking about (fig. 5).

c. Rest in bed, without sleep, apparently prolongs the emptying time over that when the individual is sitting up, but there is a somewhat earlier fall in acidity. Attention is called to the fact that this fall in acidity does not necessarily mean a fall in the potentiality curve referred to in figure 1 volume times acid divided by ten. On the contrary the potentiality curve was possibly higher.

Emptying time of the stomach with Ewald test meal at night compared with emptying time during the day. In figure 4, described above, the empty-

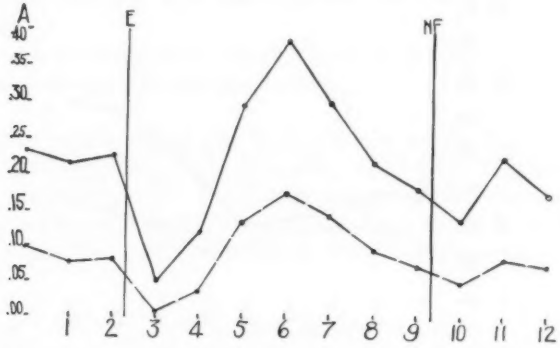

Fig. 5. A composite graph of three experiments performed in the day during the period of digestion of the breakfast meal. Intervals of fifteen minutes marked below. *A*, percentage acidity; *E*, Ewald test meal; *NF*, no food in stomach.

ing time of the Ewald meal, subject asleep, in two experiments, was $3\frac{3}{4}$ hours and 3 hours respectively. In the third experiment, subject awake in bed but relaxed, the emptying time was $2\frac{3}{4}$ hours. These results contrast strongly with those obtained with the Ewald test meal in the day during the period of digestion of the breakfast meal (fig. 5). The average emptying time for the three latter experiments was $1\frac{3}{4}$ hours derived as follows:

Experiment of 3-13-1924, emptying time $1\frac{3}{4}$ hours
Experiment of 3-20-1924, emptying time $1\frac{1}{2}$ hours
Experiment of 3-22-1924, emptying time 2 hours

Summarizing, we have average emptying time in sleep, approximately three hours, average emptying time sitting up, $1\frac{3}{4}$ hours.

Gastric secretion at night, in the waking state. Figure 6 is an average of free acidity in four experiments covering the period of maximum rise in figure 1. This control shows that the initial rise indicated in figure 1 is due to preparation for sleep and to sleep itself. The total acidity was considerably higher but ran remarkably parallel with the free acidity. The potentiality curve is shown in circles. These experiments were performed to establish the fact that the main rise in acidity in figure 1 was not due to conditions ordinarily existing at that time of day, but that they have a close relation with the condition of sleep. However, toward midnight, in these experiments, there is a remarkable tendency toward a gradual rise in acidity, free and total, including the potentiality curve. We conclude that this is due to the approach of fatigue (the tendency toward muscular

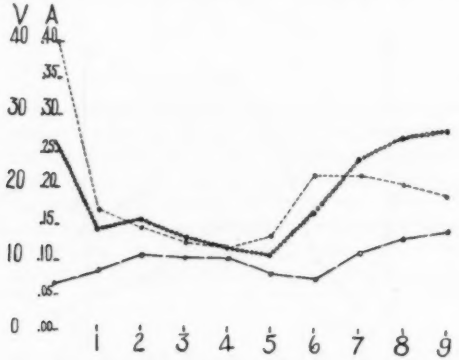

Fig. 6. Average free acidity in four experiments covering the period of maximum rise in figure 1. Intervals of fifteen minutes marked below. *A*, percentage acidity. *V*, volume in cubic centimeters. – – – – – free acidity. - - - - - - - - volume of gastric juice. o o o o o potentiality curve ($V \times A/10$).

relaxation) and the desire for sleep although these are by no means the only contributing factors. The experiments averaged in figure 6, were performed at night in the medical school laboratory to avoid the suggestion and concomitant autosuggestion of rest offered by the surroundings of a bedroom.

In spite of these conditions, however, the workers were conscious of fatigue toward the midnight hours in view of the fact that they were carrying a full research program in the working hours of the day.

SUMMARY

Fractional analyses were made of the gastric juice from a normal stomach to study the effect of sleep on the secretion curve in rest and with the Ewald test meal.

1. Preparation for sleep results in a rise in gastric acidity which continues for an hour or more after the onset of sleep.

2. This rise in acidity is generally highest when the intensity of sleep is greatest.

3. When no food is taken the gastric acidity approaches that of the resting state (normal) before midnight if the individual retires around nine or ten o'clock.

4. Sleep causes *a*, considerable delay of emptying of the stomach; *b*, a marked rise in acidity, and *c*, a delay in the fall of gastric acidity, when the Ewald meal is taken just before retiring.

5. Sleep at first accentuates then diminishes the potentiality curve of gastric juice.

6. The gastric secretion curve obtained with the empty stomach in sleep is almost identical with that obtained with the empty stomach in hypnosis (1).

7. The average volume yield of gastric juice per hour in the waking state at night was more than double that in sleep.

The authors wish to thank Drs. A. J. Carlson and A. B. Luckhardt, of the Physiology Department, The University of Chicago, for criticism and revision of the manuscript. They wish also to extend thanks to Mrs. Dowling, of 963 Heard Avenue, Augusta, Ga., for a kindly interest and cooperation while the night experiments were being carried on at her residence.

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ON THE RELATION OF BLOOD VOLUME TO TISSUE NUTRITION

VI. An Automatic and Bloodless Method of Recording the Volume-Flow of Blood

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Some years ago I described an automatic and bloodless method for recording the volume-flow of blood through a cleanly dissected vein separated from its surrounding tissue. By means of an electrically operated mechanical device the steady flow of blood through the vein is interrupted and the filling and emptying of a segment of the vein is electrically recorded.

The blood is always confined to its normal channels. It never comes in contact with foreign matter. Clotting and loss of blood do not occur. As a consequence the ordinary inconveniences and harmful effects of other continuous methods of recording the volume-flow of blood are avoided.

We have recorded the volume-flow of blood continuously for fourteen hours without the formation of clots or indication of injury to the vein. The method has proved of such great value in the various problems on the volume-flow of blood in which we have been engaged that it seemed desirable to improve the mechanism of the device not only for our own convenience but for those who may wish to employ a simple continuous method for problems of their own.

Whereas in the original arrangement three solenoids, three electrical contacts and a valve for the timing of contacts were employed, in the newer device one solenoid and one contact suffice to measure the flow of blood with greater precision.

The device consists essentially of a solenoid, *A*, mounted on an extended piece of square brass tubing, *B*, to which are attached the supporting plate, *C*, the cut-off gates, *D*, the emptying lever, *E*, and emptying plate, *F*, the electrical contact lever, *G*, with its yoke, *H*, and contact, *I*.

The vein is placed between the emptying plate and supporting plate and is held in a constant position by four upright pins, *G*, two at each end of the supporting plate. The cut-off gates are attached to a common bar, *K*, pivoted at *L* immediately back of the emptying plate. The gates are so arranged that when the outflow gate which lies nearest the heart is

closed, the inflow gate is open and the blood accumulates in the vein. When the inflow gate is closed the outflow gate is open and the blood which has accumulated in the vein beneath the emptying plate is forced on to the heart. During the filling of the vein the outflow gate is maintained in a closed position by a single turn phosphor bronze spiral spring mounted at *M*, exerting its tension through lever *N* pivoted at *M*. The tension of the spring is adjusted by screw and lock washer, *O*.

As the segment of vein fills the emptying plate rises and the free end of the emptying lever which extends into the yoke of the contact lever descends. It thereby pushes the yoke end of the contact lever down and the contact end up until the electrical contact is made and the iron core, *P*, is drawn backward into the solenoid. First the cut-off gates reverse. This is accomplished by the pull of a cord attached to the core and to lever, *N*. The blood in the vein proximal to the inflow gate is then free to flow on to the heart. Immediately after the reversal of the gates the emptying plate is drawn down upon the supporting plate by means of a second cord attached to the core of the solenoid and the right angle arm, *S*, of the emptying lever extending into the channel of the square brass tubing.

The cut-off and the emptying cords are each attached to the iron core by means of spiral springs, *T*. The purpose of these springs is to prevent jarring of the instrument, too sudden emptying of the vein, to permit the reversal of the cut-off gates before the emptying plate descends and to draw the core of the solenoid back when the circuit of the solenoid is broken thereby producing slack in both cords.

The cut-off gates are made to reverse before the emptying plate descends, so that the instrument will accurately record the filling and emptying of that portion of the vein lying between the cut-off gates. This sequence of the reversal of the cut-off gates and the descent of the emptying plate is insured by employing a spring on the cut-off cord stronger than the spring operating the cut-off gates but weak enough to permit stretching when the core of the solenoid is attracted into the solenoid. By the stretching of this spring the slack in the emptying cord is taken up and the emptying plate then descends.

Due to the inertia of the blood and to the resistance to its flow the emptying of the vein is not immediate. A short but nevertheless appreciable amount of time is required for the emptying of the vein. The emptying is insured only by the steady pull of the core of the solenoid. As the vein empties the free end of the emptying lever rises in the yoke of the contact lever until it touches the emptying screw, *X*, in the yoke of this lever. The yoke end rises and the electrical contact end descends and the circuit is broken. The magnetic pull on the core disappears, the core is drawn forward by the tension of its attached spiral springs producing slack in

the cut-off and emptying cords. The cut-off gates reverse. The outflow gate is closed by the tension of the cut-off spring and the emptying plate rests upon the emptied vein. The vein now fills and against no appreciable resistance, for there is slack in the emptying cord and the emptying and contact levers are perfectly balanced by counterweights, *B* and *W*. The cycle of the instrument is then repeated.

Since the volume-flow of blood varies under different conditions and with different animals, it is desirable to adjust the instrument to the flow of any particular experiment. If the flow of blood is rapid it is well

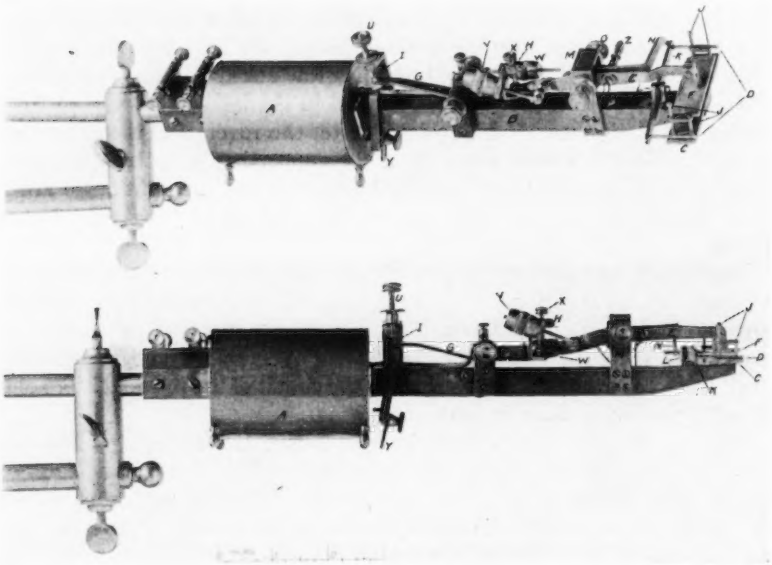

Fig. 1

Figs. 1 and 2. *A*, solenoid; *B*, square brass tube; *C*, supporting plate; *D*, cut-off gates; *E*, emptying lever; *F*, emptying plate; *G*, contact lever; *H*, yoke of contact lever; *I*, contact of contact lever; *J*, upright pins for holding vein in position; *K*, swinging bar of cut-off gates; *L*, pivot block of cut-off gates; *M*, pivot point of emptying lever and lever operating cut-off gates; *N*, lever operating cut-off gates; *O*, screw and lock washer for adjusting the spring of the cut-off gates; *P*, core of solenoid; *Q*, cut-off cord; *S*, right angle arm of emptying plate; *T*, spiral springs connecting cut-off and emptying cords to core of solenoid; *U*, filling screw for adjusting degree of filling of the vein; *V*, counterweight for balancing emptying lever; *W*, counterweight for balancing contact lever; *X*, emptying screw for adjusting degree of emptying of the vein; *Y*, iron shoe for securing firm contact; *Z*, clutch for reversing pull on the cut-off gates.

to increase the filling capacity of the vein by raising the contact or filling screw, *U*, if the flow is very slow the screw is correspondingly adjusted.

The degree of emptying of the vein is similarly controlled by adjusting the height of the emptying screw, *X*, in the yoke of the contact lever. Though complete or almost complete emptying of the vein is desired with each cycle the varying thickness of veins of different animals may require adjustment from time to time.

When the circuit is closed there is a sudden pull on the cut-off and emptying cords which tend to jar the electrical contact. As a result the contact is broken before the vein is empty and the instrument chatters. This is avoided by the adjustable iron shoe, *Y*, attached to the contact end of

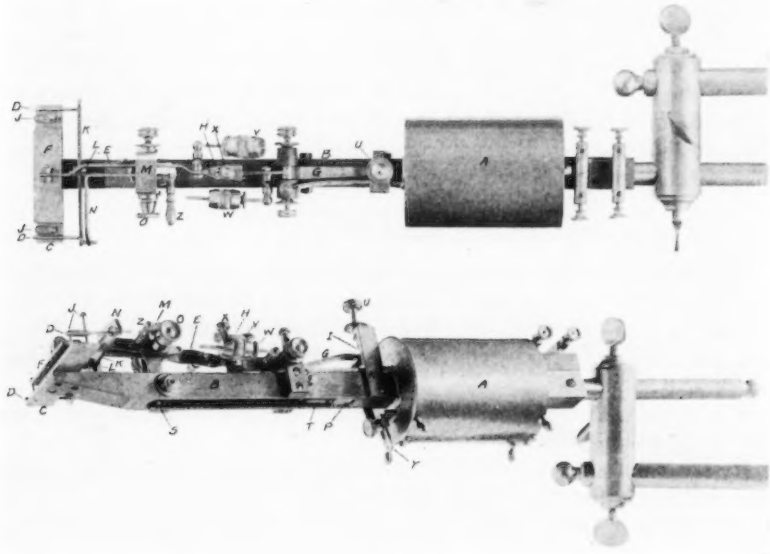

Fig. 2

the contact lever which swings around to the under side of the square brass tubing. With the shoe in proper adjustment the moment the contact is made it is firmly established by the upward magnetic pull on the shoe, and breaks only when the contact is automatically broken by the complete emptying of the vein.

It is obvious that each cycle of the instrument is composed of a large number of operations yet all of these operations are controlled by only one contact and one solenoid. The use of the instrument is therefore very simple. Once the cut-off spring (the spring which holds the outflow gate in position) and the soft iron shoe have been adjusted each following experiment requires only the adjustment of the filling and emptying screws which is a very simple procedure. It was mentioned

above that the flow of blood may be measured for hours without apparent damage to the vein. It may also be added that once the instrument is adjusted the flow of blood may be measured for a similar period without further adjustment. It is only necessary to prevent the movement of the animal and the instrument and to keep the vein in a normal moist condition. I have employed the precaution of clamping the instrument directly to the animal board.

The original instrument measured blood flowing in one direction only. The modified instrument is universal. The flow of blood may be measured in veins on either side of the body regardless of the direction of the flow. The instrument is adjusted for an opposite flow first by reversing the tension of the cut-off spring which changes the outflow and inflow gates into the inflow and outflow gates respectively and second by reversing the pull of the cut-off cord on the lever operating the cut-off gates. This pull is reversed by means of clutch Z, pivoted at M. Swinging the clutch through 180° transfers the pull from one side of the fulcrum to the other. The clutch is shown in the two positions in the photographs.

SUMMARY

A previously described automatic and bloodless method for recording the volume-flow of blood has been simplified and improved.

The blood to be measured is drained to the exclusion of all other blood into a large vein which serves as a reservoir. This vein is placed in the instrument.

The blood is then measured in the intact vein without coming in contact with foreign substance.

By means of a single solenoid and a single electrical contact the reservoir automatically fills and empties.

The flow is recorded with a signal magnet connected in parallel with the solenoid.

The advantages of the method are:

a. It is bloodless and therefore requires no anticoagulants or defibrination of the blood.

b. There is no loss of blood and therefore long experiments may be performed with a minimum deterioration of the condition of the animals.

c. The method is automatic and simple and therefore requires very little attention.

d. By calibration quantitative as well as qualitative results may be obtained.

STUDIES OF THE THYROID APPARATUS

XXVIII. The Differential Development of the Albino Rat from 75 to 150 Days of Age and the Influence of Thyro-Parathyroidectomy and Parathyroidectomy Thereon

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The eighteenth paper of this series (1) contained an outline of the principles concerned in differential development and their application to an interpretation of the rôle of the thyroid apparatus in the growth of the albino rat during the interval from 100 to 150 days of age. This paper extends the analysis into the age period from 75 to 100 days or the phase of post-pubertal adjustment.

It will be convenient to call the groups which were studied during the period from 100 to 150 days, the 100-day-old series, and those which were studied from 75 to 150 days, the 75-day-old series. This latter series is composed of 97 animals divided into ten groups; five of each sex. There are the reference controls from which the measurements at 75 days of age were obtained; the rats thyro-parathyroidectomized at 75 days of age and their unoperated litter controls, and the animals parathyroidectomized at the same age and their litter controls. The measurements on the operated groups and their controls were made when the rats were 150 days of age—75 days after the operations. The sexes were separated throughout. Diet and environment were the same for all.

A few words concerning the terminal criteria for the success of the experimental procedures seem apropos at this time.

First consider the thyro-parathyroidectomized animals. It has been repeatedly stated in earlier papers and is emphasized here that the data on which these reports are based are derived from animals in which no trace of thyroid tissue is found in the neck region by macroscopic observation at dissection. Experience has shown that such inspection is all that is sufficient and necessary. This is because the thyroid gland in the albino rat is a definitely circumscribed and encapsulated structure capable of being observed as such and of being removed with completeness and precision,

providing the field of operation is kept free from blood. Successful complete removal is a matter of knowledge of the anatomical relationships and technic. Hemorrhage is easily prevented by clamping off the superior thyroid arteries with fine clips made as described elsewhere (2). Once the structure is removed there is obviously no question of later recurrence. When such occurs technic has been faulty and removal incomplete. Residual thyroid tissue of this nature is easily detected through its difference in color from the surrounding tissues.

Although abnormality of anatomical location of the thyroid in the neck region is possible, such has never been observed in upwards of two thousand rats so far examined. This consistency of location does not prove that there is no thyroid tissue in the body other than that described. Nevertheless such has yet to be demonstrated in the normal animal.

Now the anatomical relations in the rat make futile any attempt to remove the thyroid and leave the parathyroids uncontaminated by thyroid tissue potentially capable of functional regeneration. Moreover it is improbable that transplanted parathyroids would maintain an effective functional activity throughout the long periods used in these studies. It is not necessary to go into a discussion at this time as to how far the observed effects are to be attributed to thyroid and how far to parathyroid removal. A large measure of differentiation is possible on the basis of differences in type response to the two deficiencies (thyroid-parathyroid and parathyroid). This has been done in preceding reports and a tentative interpretation outlined (3).

Such being the case it is sufficient to point out that the complete removal of the thyroid gland from its apparently normal location in the neck, together with the embedded parathyroids, produces certain distinctive and directionally uniform effects upon the growth and chemical composition of the organism, which are directly attributable to the chain of reactions set into activity by the glandular deficiency.

Consider now the parathyroidectomized rats.

All investigations of the effect of a change in environment upon a process entail a comparison of the response of a test object with its control. When the test object shows a deviation from the control, the difference is attributed to the test procedure. This is fundamental experimental practice the validity of which cannot be gainsaid. Three conditions must be observed. First, that adequate standards of control be exercised; second, that adequate methods of measurement be employed, and third, that the desired change in environment be actually produced.

In connection with these studies no discussion is needed as far as the first two conditions are concerned. The nature of the data recorded in the various papers of this series should make unnecessary any discussion

of the third condition. Nevertheless, it may be well to emphasize certain points.

All competent investigators seem to agree that in the rat there is but one parathyroid in connection with each thyroid lobe. My experience has been that there are easily discernible whitish semi-opaque spots located near the upper poles of the thyroid. They stand out clearly under the magnification used at operation. Rarely is an animal encountered in our stock, in which the parathyroids are abnormally located or too deeply embedded for satisfactory extirpation. When such are found they are discarded. Only animals with normally located glands are used, and the data which have so far been reported have been derived from those rats in which no residual parathyroid tissue was evident in the neck region or thyroid on macroscopic inspection at dissection. This might seem inadequate evidence were it not for two facts. First, that complete removal of the parathyroid by snipping is easy, and only complicated by hemorrhage when the artery clips have been insecurely placed. Second, that checks on the success of the operation are available throughout the subsequent period of observation.

There is the occurrence of tetany; there is the occurrence of teeth defects, and there is the irregularity in the growth-rate from week to week. Any or all of these may occur in one and the same animal. They have not been observed in the controls, nor in the stock as a whole, nor in the rats in which residual parathyroid tissue is found in the thyroid at dissection. Out of 32 rats parathyroidectomized at 75 days of age, 21 had defective teeth, and 15 showed definite convulsions. All but one had either defective teeth or tetany or both. The exception showed a well-defined growth irregularity which justified its inclusion in the group. The character of the dental defects has been described in an earlier paper (3). The occurrence of the above phenomena justifies the conclusion that the animals have been subjected to a definite parathyroid deficiency and their inclusion in the parathyroid groups for the study of the effects of this deficiency on growth. Further evidence that the classification is correct is given by the fact that these animals exhibit a directionally uniform deviation from the controls with respect to the growth of the body as a whole and of its parts, and in the chemical processes concerned in ossification and calcification.

According to accepted standards of experimental procedure this is all that is sufficient and necessary to demonstrate that the desired change in environment has been produced.

This is no evasion of the question of the presence of parathyroid tissue in the rat other than that found associated with the thyroid;—that is to say, the so-called accessory parathyroids. The neck region and the thymus in the rat may be riddled with little groups of parathyroid-like cells; but

a determination of this point in the rats used in this study by histological examination of serial sections means an inspection of at least four hundred and twenty thousand sections, a bit of work which I am quite willing to leave to someone else.

The matter boils down to the question as to whether or not the so-called accessory parathyroid tissue is functionally capable of taking over the work ordinarily carried on by the thyroid-associated glands. The data recorded in the various papers of this series give definite evidence that this tissue is functionally impotent. Any question as to the probability of a functionally effective compensatory hyptertrophy of this accessory tissue occurring can hardly be maintained in the face of the following facts.

Growth in body weight of the male and female rats of the 75-day-old series continued steadily but at a rate less than that of the controls for six weeks after the first shock of parathyroid deficiency. During the succeeding four weeks, growth was not only stopped but a tendency for loss of weight was exhibited (4). This should not have occurred if effectual functional hypertrophy had taken place.

Retardation of growth, tetany and defective teeth have never been observed in those parathyroidectomized rats in which residual parathyroid tissue was found associated with the thyroid at dissection. If one gland or a part of a gland is able to prevent these disturbances, why does not this mass of so-called accessory parathyroid tissue do the same?

It has been observed in many of the rats parathyroidectomized at 23 or at 30 days of age that the teeth break off, come in again apparently whole and then break off again during the four months' interval between operation and dissection. Practically all of the rats have defective teeth at the age of 150 days. One might say that the repair of the teeth after the first breakage is due to a functionally effective compensatory hypertrophy of the accessory parathyroid tissue. But if such is the case why should a second break occur, and sometimes a third? To explain this phenomenon requires the erection of an hypothesis in which waves of functional effectiveness are succeeded by waves of functional insufficiency. Perhaps this is the case. However, in view of the cumulative evidence to the contrary just given, I cannot accept the hypothesis until definite supportive evidence is presented. With this clarification let us turn to the main topic.

There are available from the records data which make possible a division of the growth interval from 75 to 150 days into two periods, one from 75 to 100, the other from 100 to 150 days of age. From the measurements which are to be found in the 18th and succeeding papers of this series, there has been calculated the increment in grams per 100 grams, and millimeters per 100 millimeters per day of the various structures for the two periods. The results of these computations are given in table 1 together

with the inter-period ratios. The values in which the 75-day-old series participate were derived from the combination of the two sets of controls. The study of this table brings out interesting relations.

The growth capacity of the various structures during the growth period from 75 to 100 days of age is greater in the males than in the females with

TABLE 1

The growth capacity (grams per 100 grams per day) of the various organs in the controls of both sexes during the two growth periods and the inter-period ratios

MALES				FEMALES			
Organ	75-100 days R_B	100-150 days R_C	$\frac{R_C}{R_B}$	Organ	75-100 days R_B	100-150 days R_C	$\frac{R_C}{R_B}$
	grams	*grams*			*grams*	*grams*	
Epididymis	6.960	1.476	21.2	Uterus	3.100	0.700	22.7
Hypophysis	2.068	0.572	27.7	Ovaries	2.880	0.226	7.8
Femur weight	2.048	0.654	31.9	Femur weight	1.760	0.480	27.3
Submax.	1.792	0.632	35.3	Hypophysis	1.684	1.200	71.3
Body weight	1.740	0.806	46.3	Adrenals	1.352	0.462	34.2
Heart	1.644	0.546	33.2	Spleen	1.224	0.370	30.2
Kidneys	1.616	0.776	48.0	Body weight	1.120	0.502	44.8
Humerus weight	1.576	0.622	39.5	Humerus weight	0.916	0.492	53.7
Lungs	1.252	0.600	47.9	Heart	0.816	0.384	47.1
Spleen	1.120	0.640	57.1	Submax.	0.782	0.422	54.0
Testes	1.000	0.434	43.4	Spinal cord	0.760	0.402	52.9
Pancreas	0.976	0.502	51.4	Lungs	0.680	0.402	59.1
Eyeballs	0.816	0.314	38.5	Eyeballs	0.668	0.278	41.6
Spinal cord	0.812	0.476	58.6	Kidneys	0.628	0.408	65.0
Liver	0.788	0.460	58.4	Liver	0.336	0.356	106.0
Adrenals	0.416	0.210	50.5	Pancreas	0.336	0.294	87.5
Brain	0.148	0.144	97.3	Brain	0.040	0.118	295.0
	mm.	*mm.*			*mm.*	*mm.*	
*Femur length	0.512	0.274	53.5	Femur length	0.368	0.206	56.0
Humerus length	0.416	0.256	61.5	Humerus length	0.280	0.220	78.6
Tail length	0.416	0.170	40.8	Body length	0.244	0.220	90.2
Body length	0.400	0.250	62.5	Tail length	0.192	0.153	69.8

*The length values are obviously in millimeters.

the exception of the spleen, spinal cord, gonads and adrenals. The two former show a sex-difference which at present can be considered as negligible. This sex similarity in growth ability at this time marks these organs out as distinctive, an explanation for which is not at hand.

The adrenals and gonads also exhibit a distinctive sex-difference in growth capacity as compared with the other organs of the body. The

rate of growth of both ovaries and adrenals is greater than that of the testes and adrenals during this period of post-pubertal adjustment. This may be an expression of a sex-conditioned gonad-adrenal inter-relationship. This idea is supported by studies of the comparative embryology of the gonads and the adrenals and by the results of physiological experimentation. One the one hand Soulié (5), in an exhaustive study of the development of the adrenal glands in invertebrates, found that the anlage of these

TABLE 2

The growth capacity of the various structures in the females in terms of that of the males

$$\frac{\text{♀ G.C.}}{\text{♂ G.C.}}$$

ORGANS	RELATIVE GROWTH CAPACITY	
	75-100 days	100-150 days
Adrenals	325	220
Testes-ovaries	288	52
Spleen	109	58
Spinal cord	94	84
Femur weight	86	73
Eyeballs	82	89
Hypophysis	81	210
Body weight	64	62
Humerus weight	58	78
Lungs	53	67
Heart	50	70
Epid.-uterus	45	47
Submax.	44	67
Liver	43	77
Kidneys	39	53
Pancreas	34	59
Brain	27	82
Femur length	71	75
Humerus length	67	86
Tail length	59	78
Body length	48	88

structures is intimately associated with the germinal epithelium. On the other, Riddle (6) has demonstrated that ovulation is accompanied by adrenal hypertrophy in the pigeon. These several findings are suggestive, but further analysis must await additional data. It should be noted that the decrease in growth capacity of the ovaries for the growth period from 100 to 150 days as compared with the period from 75 to 100 days is much greater than is that for the testes, and that the cut in adrenal growth capacity is also greater in the females than in the males.

The growth capacity of the several structures during the growth period from 100 to 150 days is also greater in the males than in the females with the exception of the adrenals and the hypophysis. The significant point at this time is the fact that the growth capacity of the male organism is generally greater than that of the female during both of the growth periods under discussion.

This superiority of the males is, however, not the same in degree in the two periods. Table 2 gives the growth capacity of the several organs in the females in terms of that in the males as calculated from the values in table 2. An inspection of the table shows that the sex-differences in growth capacity are generally greater during the period of post-pubertal adjustment than during adult growth. The magnitude of both the superiority and inferiority of the males is greater during the former than during the latter period. The implications of this finding are many, but can best be developed when the complete age data are available. Turning from the inter-sex to the inter-period comparison certain generalizations are possible.

Table 1 shows that the growth capacity of the several organs is less in both sexes during the growth period from 100 to 150 days than during the period from 75 to 100 days. The brain in both sexes and the liver in the females are exceptions. This is an expansion of the well-known fact that the rate of growth decreases with advancing age. This decrease in growth capacity is not uniform in degree for the different organs. There is a general tendency for those structures which have a high growth capacity in the earlier period to show a greater percentage reduction in the later period, and for those structures which have a low growth capacity in the earlier period to show a lesser percentage reduction in the later period. The regularity of the expression of this relation is not great but is sufficiently marked to be considered. The phenomenon is what might be expected from the fact that most of the individual organs exhibit a logarithmic form of growth curve as is shown from the graphs in Donaldson's book, *The Rat* (7), and Hatai's formulae therefor.

This relative difference in degree of decrease in growth capacity with age is exhibited in the inter-sex as well as in the inter-organ differences in one and the same sex. The degree of decrease in growth capacity is generally greater in the males in just those organs in which the superiority of the males in growth capacity over the females is greater in the growth period from 75 to 100 days than in the growth period from 100 to 150 days of age: less in those in which the male superiority is less in the first than in the second growth period, while practically no sex difference is shown in those structures in which the sex-differences in growth capacity are the same for the two periods. Certain exceptions are found, but the general picture is as stated. It would seem from this as if the two sexes are

bound by the possession of a common intensity factor of growth probably specific in origin.

It is clear from these data that the various organs differ among themselves in their respective growth capacities at a given age; that the growth capacity of one and the same organ differs at different ages; that the degree of change in growth capacity on age differs for different organs, and that sex-differences exist in the expression of the above relations. Some idea of the complexity of the factors at work in differential development are

TABLE 3

The relative growth capacity of the thypar and parathy groups of the two series in terms of that of their controls

THYPARS (G.C.)					PARATHYS (G.C.)				
Organ	Males		Females		Organ	Males		Females	
	75 day	100 day	75 day	100 day		75 day	100 day	75 day	100 day
Hypophysis	119.6	162.2	106.1	66.7	Submax	128.0	130.4	221.8	209.4
Eyeballs	83.4	82.5	88.8	50.4	Eyeballs	74.6	100.6	87.6	75.5
Epididymis (uterus)	69.5	65.0	29.7	−107.7	Epididymis (uterus)	67.0	97.2	34.9	−31.1
Testes (ovaries)	59.6	51.2	42.3	−236.3	Spinal cord	59.6	90.8	80.8	87.6
Humerus weight	57.2	27.8	42.9	−2.8	Testes (ovaries)	43.5	109.2	50.5	−90.3
Spinal cord	56.9	51.9	61.0	53.7	Hypophysis	42.4	58.4	59.2	60.2
Femur weight	54.4	33.6	37.2	11.7	Femur weight	40.2	67.0	71.4	42.9
Lungs	47.1	−21.9	38.7	−47.8	Lungs	38.8	25.7	57.5	80.6
Body weight	39.9	31.0	17.6	−28.2	Humerus weight	38.6	59.5	77.6	39.4
Brain	34.0	20.5	17.1	3.4	Heart	27.2	76.9	39.5	78.2
Submax	25.8	0.6	12.9	0.5	Brain	17.6	108.3	74.2	89.8
Heart	23.8	−26.7	24.1	−48.4	Spleen	17.3	139.1	49.4	128.1
Kidneys	15.7	−39.1	−20.7	−82.8	Body weight	13.7	57.1	30.1	20.7
Adrenals	6.2	−123.0	0.0	−124.7	Pancreas	4.8	86.5	25.9	72.8
Pancreas	−4.9	−42.0	−86.2	−124.5	Kidneys	2.0	33.8	5.2	23.5
Spleen	−19.2	−38.1	−32.5	−121.1	Liver	−26.1	63.0	−3.9	58.6
Liver	−21.3	−43.5	−28.4	18.4	Thymus	−32.8	−46.3	−2.9	−321.2
Thymus	−30.8*	−157.2	−3.9*	−556.2	Adrenals	−52.9	18.1	18.8	−3.9
Thyroid					Thyroid	−282.9	63.9	−423.3	47.3
Humerus length	49.3	42.2	40.5	25.4	Femur length	50.7	73.7	59.9	59.2
Femur length	48.5	42.3	49.7	27.2	Tail length	46.3	63.9	63.6	49.3
Body length	43.1	35.2	41.9	24.5	Humerus length	44.9	70.3	47.1	54.5
Tail length	38.4	37.2	30.4	19.4	Body length	39.4	64.0	50.0	47.2

*Values are direct multiples; not percentages.

brought out by this analysis. One or two classifying principles are beginning to emerge from the tangle, they are of assistance in interpreting the effects of the glandular deficiences now to be discussed.

Table 3 gives the relative growth capacities of the various structures in the rats of the operated groups in terms of those of their controls for the two age series observed.

The table shows that the retardation and retrogression effects of a thyroid (thypar) deficiency were less marked in both sexes in the 75 than in the 100 day old series. That is to say, the initiation of a thyroid (thypar) deficiency at 75 days of age produces in general a lesser disturbance

of growth during the subsequent interval of 75 days, than does the initiative of a like deficiency at 100 days on growth during the subsequent 50 day interval.

There are three factors to be considered as possible participants in this differential response. They are: the duration of exposure to the glandular deficiency; the stage of physiological development at the initiation of the deficiency, and the strength of the growth capacity at the time of glandular extirpation. The last two are, of course, intimately related in the intact animal, while all three are inter-reacting in the defective individual. It is worth while to attempt an evaluation of the relative importance of these factors in the response.

A longer duration of exposure might be expected to produce a greater disturbance of growth through the cumulative effect of the deficiency, or to allow a greater degree of recovery and hence an apparent lesser disturbance. The former is evidently not of significance with the possible exception of the thymus in the males, and the liver and hypophysis in the females. Even where the retardation effect was practically the same in both age series (as in the eyeballs, epididymis, spinal cord and tail in the males) the direction of difference was toward a lesser retardation in the animals operated at the earlier age. The probability that recovery due to the longer period of exposure is a large factor in the generally better growth in the thypars of the 75 day old series is small, since, as was shown in an earlier paper (4) the initial disturbance in growth in body weight in these rats was much less than that observed in the rats deprived of the thyroid apparatus at 100 days of age. That is to say, the immediate resistance of the animals to thyroid (thypar) lack is greater at 75 than at 100 days of age.

The relation of the stage of functional development of any particular structure to the call of the organism as a whole on its function; the relation of this to the growth and size of the structure; the relations of these to the combined influences of the functional activities of all the other organs, and the peculiar influence arising at puberty must all be considered as participants in the determination not only of the strength of the growth capacity of any organ, but also of the specific degree of its response to thyroid deficiency initiated during the period of post-pubertal adjustment.

If we agree that the gonads are factors of special influence at this time, and note that the testes apparently are more mature than the ovaries (from the fact that the growth capacity of the testes is decreasing much less rapidly than that of the ovaries, table 1), it can be assumed that the males at 75 days of age have reached a higher degree of stability with respect to the influence of the pubertal changes than have the females. If such is the case, it is probable that the post-pubertal phase of development plays a lesser rôle in the subsequent growth of the males than of

the females, and consequently a lesser rôle in the response to thyroid (thypar) deficiency. A certain degree of justification for this assumption is to be had from the analysis of the gross growth of these animals (4).

This same analysis showed that the greater resistance of the growth in body weight in the animals operated at 75 days of age to the growth inhibiting effects of thyroid (thypar) deficiency, is in large part attributable to the greater strength of the growth capacity at the earlier age. Such being the case, and since the various structures show the same greater growth capacity at 75 than at 100 days of age, and since, as noted previously (1), the reduction in growth capacity caused by thyroid (thypar) deficiency tends to be negatively correlated with the normal expected growth capacity of the individual organ, I believe that the greater resistance of the various structures in the animals operated at 75 days of age to the growth inhibiting effects of thyroid (thypar) deficiency is largely due to the greater strength of their growth capacities at the earlier age.

Continuing the inter-series comparison it is seen from table 3 that the lack of thyroid (function) has resulted in a disproportionate differential development in the 75 as in the 100 day old series. This distortion is qualitatively similar in the males of both series, with the exception of the lungs, adrenals and humerus weight. That is to say, the initiation of a thyroid (thypar) deficiency in male rats 75 days of age produces in general the same type of distortion of differential development as does the initiation of a like deficiency at 100 days. In the females the response is otherwise. There is a widespread deviation of the distortion from that exhibited in the 100 day old series. That is to say the distortion of differential development in the females after thyro-parathyroidectomy at 75 days of age is different from that which follows the same procedure at 100 days. This sex-difference is attributable to the difference in state of maturity of the gonads as has been already discussed.

As in the 100 day old series so in the 75 it is clear that the extent of the disturbance of development in terms of inter-organ comparison bears no relation to the normal growth capacity.

Retrogression was less widespread in the animals operated at the earlier age, yet the organs primarily concerned with the vegetative functions of the body are seen to be the more deeply affected here as in the groups deprived of the thyroid apparatus at 100 days of age. This consistency of response supports the hypothesis previously expressed that the general retardation of growth in thyroidless animals is as much a consequence of an inadequate preparation and presentation of metabolites to the tissues as of a general lowering of the plane of cell metabolism.

Turning now to the inter-sex comparison it is seen from table 3 that in the 75 as in the 100 day old series the decrease in growth capacity caused by thyroid (thypar) deficiency is generally less in the males than in

the females. This sex difference is less in degree in the rats operated at the earlier age. In addition the impression is given that the two sexes differ less in the nature of the distortion of differential development after thyro-parathyroidectomy at 75 days of age than they do after the same procedure at 100 days.

This drift toward approximation of the two sexes in the degree and kind of response may be due to the fact that complete sex maturity is not yet attained at 75 days of age and hence the sex conditioning of the differentiation of response to the glandular deficiency is less in force. This conception is supported by the marked sex-difference on age of the reaction of the gonads to thyroid (thypar) deficiency.

In view of the rôle of the hypophysis in growth, attention should be called to the sex-difference on age in the response of this organ to thyroid (thypar) lack. The significance of the alteration is not clear at the present time, but its occurrence is none the less noteworthy.

So much for the effects of thyroid (thypar) deficiency.

The values in table 3 show that quite a different story is written when parathyroid function is deficient. In the first place the retardation or retrogression of the growth of all structures in the males (save the lungs) was greater after parathyroidectomy at 75 than at 100 days of age, while in the females a mixed response obtained. Notwithstanding the fact that the growth in body weight as a whole was less retarded by the toxemia of parathyroid deficiency initiated at 75 days than by the same disturbance initiated at 100 days, many of the organs in the females as in the males (brain and spinal cord, heart, lungs, spleen, pancreas, kidneys, liver and thyroid) were retarded more in the groups subjected to the influence at the earlier age.

In the interpretation of this phenomenon it is necessary to consider the same three factors as were discussed in connection with the results following removal of the thyroid apparatus as a whole.

While the strength of the growth capacity at the time of removal of the parathyroids may be a factor it is probable that its participation in the final picture is of less significance than either the stage of physiological development or the duration of exposure to the toxemia.

This opinion is founded on the fact that the degree of growth disturbance in the males was consistently greater in the 75 than in the 100 day old parathy series, the opposite of that exhibited in the thypars, and the fact that the same direction of difference was exhibited in the significant organs in the females. If growth capacity *per se* is of preponderant importance in the sense of opposing the growth inhibiting influence of parathyroid deficiency, the retardation of growth should have been generally less after parathyroidectomy at 75 days of age. This opinion is strengthened by the fact that the disturbance of growth of the 75 day old male para-

thys was greater, organ for organ, than was that of the females of the same series group.

Since this difference is the opposite from that exhibited by the parathys of the 100 day old series it is evident that the age differences in degree of response are sex-conditioned. This belief is supported by the fact that at 75 days of age the male albino rat is more sensitive to the toxemia of parathyroid deficiency than is the female and that the sex difference tends to be less in the groups operated at 100 days. This implies that the stage of physiological development in relation to sexual maturity is a factor of importance in the determination of the quantity response to the toxemia. Support for this assumption is had in the marked sex-difference on age of the reaction of the gonads to parathyroid deficiency.

The longer duration of the exposure to the toxemia is also a factor in the greater retardation of growth of the various parts in both sexes after parathyroid removal at 75 days of age. This is probable from the fact that the gross growth of the parathys in body weight was not only completely inhibited but also caused to retrogress in the later weeks of the period of observation. Such an interpretation is not incompatible with the belief that the stage of physiological development is a factor, since this would obviously, through its influence during the early stages, be a partial determinant of the subsequent response to the cumulative action of the toxic products.

It is evident from table 3 that the induction of a parathyroid deficiency at 75 days of age produces a distortion of differential development in both sexes which is quite different from that produced by a like procedure at 100 days; that this distortion differs in the two sexes, and that both sexes differ in this respect from the thypars. This is all that can be said at this time along this line because the chaotic nature of the distortion precludes any satisfactory analysis. The point should not be overlooked, however, that thyroid (thypar) deficiency is accompanied by a distortion of differential development which is distinct in degree and kind from that which accompanies parathyroid deficiency.

In order to save space no table is given of the coefficients of variability of the several groups. It is sufficient to state that the initiation of the glandular deficiencies at 75 days of age was, in general, followed by the same increase in variability as was found to occur after thyro-parathyroidectomy and parathyroidectomy at 100 days.

SUMMARY AND CONCLUSIONS

The growth capacity of the several organs of the normal male albino rat during the period of post-pubertal adjustment from 75 to 100 days of age, is in general greater than that of the female as it is during the period

of more adult growth from 100 to 150 days. The magnitude of the sex difference is greater during the first than the second growth period.

The growth capacity of the individual organs of both sexes decreases during the growth period from 75 to 150 days of age, and the decrease is roughly inversely proportional to the initial value.

Disproportionate differential development in both sexes follows the removal of the thyroid apparatus at 75 as at 100 days of age. In the females of the 75-days-old series the nature of the distortion differs from that of the 100-day-old series. In the males the type response is closely similar in both age groups.

In both sexes those organs which are primarily concerned with the vegetative functions of the body are among the ones most seriously disturbed. This confirms the findings and interpretation previously made on the thypars of the 100-day-old series.

The initiation of a thyroid (thypar) deficiency at 75 days produces a lesser retardation of growth of the several organs in both sexes, than is produced by the same procedure at 100 days. This is largely attributable to the greater initial strength of the growth capacity in the younger animals. The longer period allowed for recovery and the stage of physiological development (age) are possible participating factors.

The decrease in growth capacity due to thyroid (thypar) deficiency is less in the males than in the females in the 75 as in the 100-day-old series. The magnitude of the sex difference tends to be less in the former than in the latter.

The toxemia of parathyroid deficiency causes a disproportionate differential development in both sexes, which differs for the two sexes, and differs from that exhibited by the 100-day-old series.

The males are uniformly more sensitive to the toxemia following parathyroid removal at 75 days than the females. This is shown by the greater general retardation of growth, a reaction opposite to that exhibited in the 100-day-old series.

In the males the retardation of growth of the several organs is greater after parathyroidectomy at 75 days than after the same procedure at 100 days. In the females the same is true of the brain, spinal cord, heart, lungs, spleen, pancreas, kidneys, liver and thyroid. The other structures in this sex are either less retarded or show no age difference.

The greater retardative effect of parathyroid removal at 75 days of age is largely attributable to the cumulative effect of the longer periods of exposure to the toxemia of the glandular deficiency. Sex specific factors undoubtedly contribute to the determination of the sex differences in response on age.

As in the 75, so in the 100-day-old series, a general increase in organ variability is produced by the glandular deficiences.

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THE PRODUCTION OF ANHYDREMIA WITH INSULIN[1]

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The condition of hypoglycemia has been produced in several ways. Especial interest, however, centers in the hypoglycemia following the use of excessive amounts of insulin because of its bearing upon the use of a very important therapeutic agent and because of the desirability of studying an interesting "symptom complex" (1) resulting from an apparent failure of several functional mechanisms.

Blood dilution and anuria have been observed by Fisher and Wishart (2) to succeed the state of hyperglycemia due to the ingestion of large amounts of glucose. In a series of studies, Barbour and co-workers (3) have demonstrated that, in fever, glucose exerts an antipyretic influence and that the basis of antipyretic action is a hydremic plethora due to hyperglycemia. From these studies it appears that, under certain conditions, an increase in blood sugar causes a mobilization of water into the blood. That the antithesis of hypergylcemia and plethora, namely, a virtual dehydration of the blood in certain hypoglycemic states, is also true seems to be indicated by the recent work of Underhill and Karelitz (4) in hydrazine poisoning.

The symptoms in marked insulin hypoglycemia present many features suggestive of the condition of traumatic shock. Of interest in this connection are the observations of Gasser, Erlanger and Meek (5) on the problem of shock, in which they point out that blood concentration is the most common finding. Observations upon human subjects further suggest the possible association of anhydremia with insulin hypoglycemia. Fletcher and Campbell (1) find that in adults the outstanding "hypoglycemic reaction" is a profuse sweat, while in children it is a change in pulse rate. Joslin, Gray and Root (6) have made the interesting observation that children and adults weakened and desiccated, especially by diarrhea, are prone to develop dangerous hypoglycemia even with small amounts of insulin.

[1] A preliminary report of this work appeared in the Proc. Soc. Exper. Biol. and Med., 1924, xxi, 309.

Since the publication of our preliminary report Olmsted and Taylor have published a report in this JOURNAL (1924, lxix, 142) confirming certain of our findings on the concentration of the blood in insulin hypoglycemia.

In view of these considerations—the association of hyperglycemia and plethora, the observed symptoms suggestive of anhydremia—we were led to study the concentration of the blood in insulin hypoglycemia.

Experimental procedure. The experiments were performed upon dogs. In most instances the animals were anesthetized with iso-amyl-ethyl-barbituric acid given intraperitoneally in doses ranging from 50 to 60 mgm. per kilo body weight. Previous observations (7) have indicated that this drug has little influence on the blood sugar level or the blood pressure. In several control experiments we have shown that this anesthetic has little effect upon the blood concentration.

The blood samples for test purposes were drawn mainly from the external jugular and the femoral veins. After obtaining the control sample a large dose of insulin (20 units per kilo body weight) was injected intravenously. Following the insulin, additional blood samples were taken, the first about one hour after the administration of the insulin, and usually four or five other samples at approximately one-half hour periods. The blood sugar was determined quantitatively by the method of Shaffer and Hartmann (8) and the hemoglobin, as the index of blood concentration, by the method of Cohen and Smith (9).

A record of mean blood pressure was taken at frequent intervals during the course of each experiment. In some of the experiments the proportion of corpuscles to plasma in the normal and in the hypoglycemic state was determined by centrifuging blood samples in graduated tubes. In one experiment the plasma and total blood volumes before and after insulin were determined by the dye method of Keith, Rowntree and Geraghty (10), and red blood cell counts were made with the Zeiss-Neubauer hemocytometer. In all experiments observations were made upon apparent changes in certain physical properties of the blood, such as the viscosity, tendency to clot and changes in color.

The experimental results. Venous blood, taken from the jugular, femoral or vena cava at suitable intervals after large doses of insulin, shows that with a falling blood sugar percentage there is an increase in blood concentration, as indicated by a rise in the percentage of hemoglobin. The increase in hemoglobin ranges in our experiments from values as low as 14.5 per cent to as high as 44.2 per cent, the corresponding blood concentrations being 15.1 per cent and 44.8 per cent. In table 1 the main results of this group of experiments are presented.

Peripheral vasoconstriction, with resulting peripheral stagnation, is advanced by Utheim (11) and Marriott (12) as an explanation of the greater concentration found in the capillary than in the venous blood of athreptic infants. In one experiment we have taken blood samples simultaneously from the femoral vein and from the vena cava at a point just above the renal veins. The concentration of the cava blood was found to be 12.7 per cent more than that of the femoral (44.8 per cent as compared with 32.1 per cent).

The values for blood sugar were 0.047 per cent in the cava and 0.07 per cent in the femoral vein. These data are insufficient to warrant definite conclusions, but they are of interest in this connection in that they indicate a greater concentration and a correspondingly lower percentage of blood sugar in venous trunks which are remote from the capillary blood bed.

Although there are observed differences in the degree of blood concentration in different parts of the circulatory system, yet it will be seen from table 1 above that a concentration change was present in all parts of the venous system studied. The degree of concentration seems to depend not so much upon the vessel from which the blood is drawn as upon the individual reaction to the same set of experimental factors.

TABLE 1

NUMBER OF EXPERIMENT	VESSEL TAPPED	BLOOD SUGAR		HEMOGLOBIN		LENGTH OF TIME AFTER INSULIN		INCREASE IN HEMOGLOBIN	BLOOD CONCENTRATION	REMARKS
		Before insulin	After insulin	Before insulin	After insulin					
		per cent	*per cent*	*per cent*	*per cent*	*hr.*	*min.*	*per cent*	*per cent*	
1	Jugular	0.140	0.038	98.6	115.5	2	10	16.9	17.1	
2	Jugular	0.138	0.053	95.8	110.3	2	35	14.5	15.1	
3	Jugular	0.134	0.012*	83.5	106.4	1	24	22.9	27.4	
5	Jugular	0.120	0	121.9	163.0	2	37	41.1	33.7	
6	Femoral	0.140	0.022	100.7	126.0	3	5	25.3	25.1	
7	Femoral	0.090	0.070	101.4	133.9	2	3	32.5	32.1	Blood simultaneously from femoral and cava
	Vena cava	0.081	0.047	98.7	142.9	2	7	44.2	44.8	
8	Femoral	0.100	0.012	117.2	129.3	5	50			Unanesthetized dog
			0.045†		138.8	6	12	21.6	18.4	
9	Femoral	0.124	0.030	98.2	117.0	1	42	18.8	19.1	
11	Femoral	0.136	0.022	104.2	140.8	1	34	36.6	35.1	

*Sugar down to zero 20 minutes later.

†Blood taken after most severe convulsion.

The relation of blood concentration to blood sugar level and mean blood pressure is typically portrayed in the graphic record shown in figure 1. The curve of blood sugar shows a steep fall during the first hour and one-half after insulin, then a more gradual one. The percentage of hemoglobin rises simultaneously, with the steepest gradient of the curve shown during the second hour. This record seems to indicate that there is first a fall in blood sugar, then after a short delay blood concentration takes place. The mean blood pressure tracing shows a slight decline during the first two hours after insulin, then a tendency toward a gradual return to normal. This graph is also typical of most of the experiments in that the hemoglobin and blood pressure curves show a striking similarity of contour in opposite directions.

The influence of intravenous injections of glucose subsequent to the production of the hypoglycemic state was studied in some experiments. In figure 2 are presented the results of one experiment typical of this group.

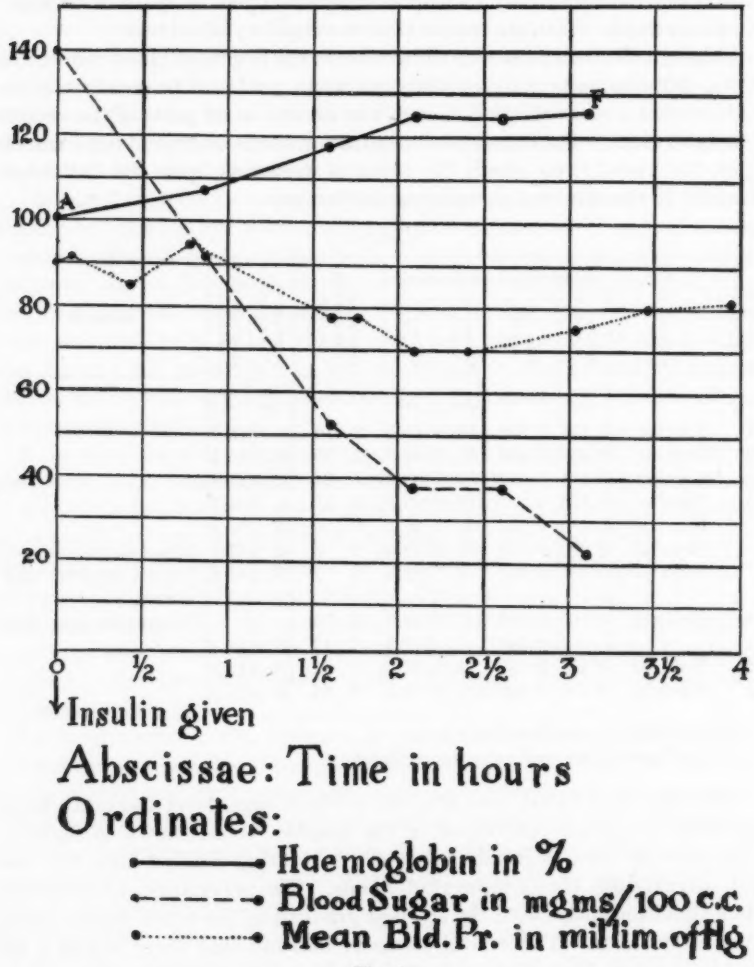

Fig. 1

This animal was rendered hypoglycemic with insulin in the usual way, and at the end of two hours received an injection of 0.65 mgm. of glucose per kilo body weight. The curves show that following this injection there was

an immediate fall in the percentage of hemoglobin, a slight but sudden rise in blood pressure and a rise in blood sugar. The curve for blood sugar shows also that at the end of about one hour after the administration of the

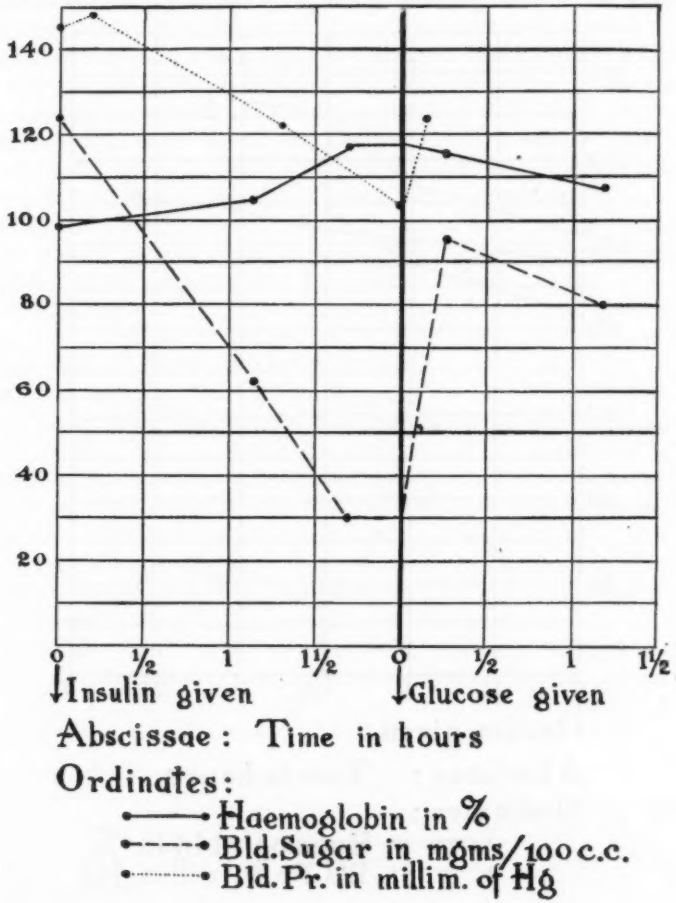

Fig. 2

glucose there is a slight decrease in the level of this substance in the blood. This feature of the curve probably indicates a utilization of the injected sugar by some remaining insulin.

An analysis of the relationship of blood concentration to mean blood pressure yields results that may be arranged under two main groups. In

figure 3 are presented graphically the data of three experiments to illustrate these contrasting types of response. The curves designated *2* characterize very well the first group. They show that the alterations in blood concentration and in blood pressure in this group are only moderate in degree

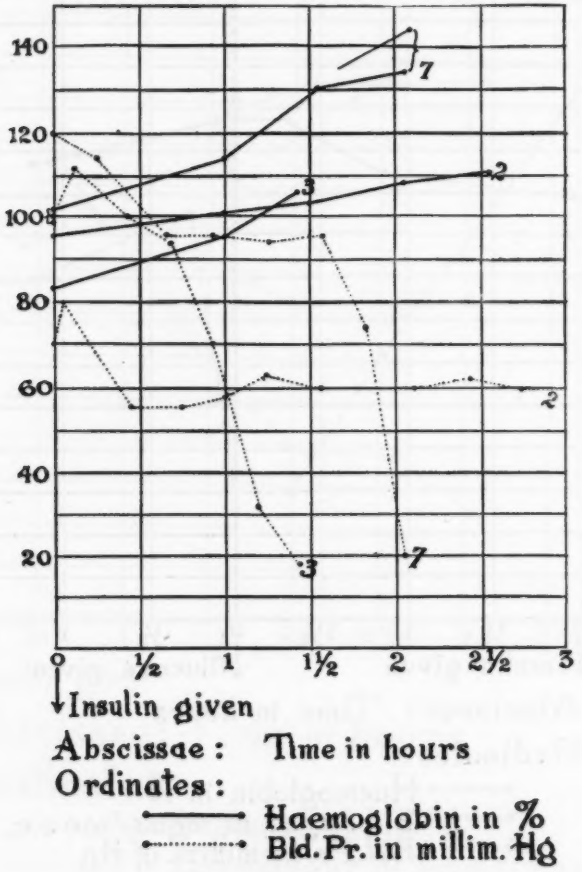

Fig. 3

but practically uniformly progressive. The second group is set forth typically in the graphs designated *3* and *7* of the same figure. The features of interest in this type of reaction are the comparatively steep rise in the curves representing the percentage of hemoglobin and the sudden transition in the record of blood pressure from a slow decline in the early part of the experiment to a rapid fall, which continues to a fatal level. It is of interest

to note in this connection that in certain experiments of our series which presented this form of circulatory collapse the blood samples had a sugar content too low to admit of detection by the Shaffer-Hartmann method.

The experimental results indicate that in general blood concentration is closely associated with failing circulatory dynamics. The changes usually proceed in a manner which suggests a common factor in their production. An apparent exception to this rule, however, is presented in the results of one experiment in which a marked concentration of the blood developed in the first hour after the insulin was given, but blood pressure was maintained at practically the original level for over three hours. In the subsequent hour a fall of 56 mm. Hg was recorded and the usual symptoms of circulatory failure supervened. The main interest of the observation lies in the possible bearing it may have upon the rôle of blood concentration in initiating circulatory failure. The fact that these results indicate an essential stability of circulatory dynamics, notwithstanding the relatively great decrease in blood volume, gives additional proof of the great adaptability factor of the cardio-vascular system.

Indirect evidence of the concentration of the blood in insulin hypoglycemia was obtained from the visible changes in the physical qualities of the blood samples. The specimens with high hemoglobin and low blood sugar concentrations showed a very apparent increase in viscosity, a marked tendency to clot and a much darker color of the venous blood suggestive of anoxemia. Changes of a similar nature have been reported by Keith (13) in experimental dehydration. In our experiments the degree to which these changes were exhibited in the blood samples appeared to have some relation to the amount of increase in percentage of hemoglobin.

The proportion of plasma to corpuscular volume was found to be disturbed, the percentage of plasma being relatively reduced in insulin hypoglycemia. An indication of this type of change is very well brought out in the results of two experiments presented in tabulated form below:

NUMBER OF EXPERIMENT	PLASMA		HEMOGLOBIN		BLOOD CONCENTRATION	REDUCTION IN PLASMA
	Before insulin	After insulin	Before insulin	After insulin		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
9	56.3	49.5	98.2	117.0	19.1	12.0
11	58.3	46.9	104.2	140.0	35.1	19.5

In experiment 9 the plasma change is 62.8 per cent of the change in concentration, and in experiment 11 it is 55.5 per cent. The apparent increase in cellular bulk exhibited by the data above is probably not due to a swelling of the corpuscles by the imbibition of water but to the loss of plasma or of water from plasma. In support of this view are the results of red

blood cell counts in experiment 11 which gave a value of 7,600,000 before insulin and of 10,700,000 after the production of hypoglycemia. In this experiment we also determined the effect of hypoglycemia upon the plasma and total blood volumes. The results of this determination, which are tabulated below, indicate that there is a reduction in total volume and that relatively the greater reduction is in the plasma volume.

WEIGHT OF DOG	PLASMA		TOTAL BLOOD		PERCENTAGE OF REDUCTION	
	Before insulin	After insulin	Before insulin	After insulin	In plasma	In total blood
kgm.	*cc.*	*cc.*	*cc.*	*cc.*		
5.25	313.4	146.8	541.3	313.0	53.1	42.1

These results, while clearly indicating a greater alteration in the plasma part of the blood, probably do not exhibit the exact magnitude of the change. The blood volume was determined by the vital red method (10) with an interim of 52 hours between the first test for normal blood volume and the second test made just subsequent to the production of hypoglycemia by insulin. This interval of time proved insufficient for complete disappearance of the dye from the blood and, as a result, the second readings were not obtained with accuracy. When due allowance is made for the errors inherent in the method the data are still valuable, we believe, in indicating that the nature of the blood volume change in insulin hypoglycemia is a significant reduction in the quantity of plasma.

Discussion. There is some indication that the reduction of a hyperglycemia to normal has not the influence upon blood concentration that we have found to be the constant outcome of the production of hypoglycemia with large amounts of insulin. Banting and Best (14) report no change in the percentage of hemoglobin in one experiment in which the blood sugar was reduced from 0.35 to 0.09 per cent, and Straub, Gunther and Fröhlich (15) reduced the blood sugar of a patient in diabetic coma to the normal level in four and one-half hours by injecting 200 units of insulin and found no accompanying change in the hemoglobin.

In our experiments in which the dosage was fixed individual reaction—predisposing factors in the animal itself—appears to have been the main determinant of the type of blood concentration response. The true nature of the predisposing factors in our animals is not evident from the data at hand. In this connection it is of interest to note that in insulin therapy in human subjects some of the factors rendering the patients especially susceptible to collapse are hypersensitiveness, cardiac or renal disease and debilitated or marasmic states often accompanied by desiccation (6). The last seems to be the most important single predisposing factor. Interest in this observation lies in the meaning given to it by the evidence presented above on blood dehydration.

The disturbances in functional equilibrium resulting from overdoses of insulin are commonly varied in their manifestations and often quite severe in character. These features, with other more direct evidence, clearly suggest a disordered action on the part of several important functional mechanisms. Indeed this idea is indicated at present in the use, by some writers, of such expressions as "hypoglycemic reaction," "symptom complex" of hypoglycemia, and "insulin shock." A considerable degree of hypoglycemia seems to be an important initial condition for the development of the group of physiological changes that attend the use of too much insulin; nevertheless, it appears that some of the alterations, e. g., certain vascular changes following a long-continued action of excessive amounts of insulin, are only secondarily related to the blood sugar condition. Moreover, the term *hypoglycemia* emphasizes the blood condition but does not necessarily denote actual functional derangement of the organism. In view of these considerations it seems desirable to give a special designation to the set of functional changes accompanying excessive insulin action which, in part, consists of a blood concentration, a greater water output (increased perpiration), a reduced cardio-dynamic capacity, and disturbances in the nervous system. Since it is desired to stress the fact that associated with the over-insulin action there is a general upset, or bad functional condition of the body, the term *hyperinsulin dyshexia* is proposed to denote this particular type of physiological disturbance.

An outstanding property of the animal body is the power of adaptability to changed conditions within itself. There is evidence to indicate that the living organism can accommodate, to a certain extent, for changes in the blood sugar level. As pointed out in the earlier part of this paper, a suddenly formed hyperglycemia results in a temporary blood dilution, but Keith, Rowntree and Geraghty (10) have found, on the other hand, that in chronic hyperglycemia there is no increase in blood volume. Marriott (16) reviews the work of E. F. Adolph and others with the statement, "When sudden loss of water from the body occurs the composition of the blood is more affected than when the same loss is brought about more gradually." In certain of our experiments dealing with the relation of blood pressure to blood concentration evidence was presented to show that the element of time, that is, the rapidity with which the blood concentration develops, plays an important part in the development of some of the signs associated with insulin hypoglycemia.

SUMMARY AND CONCLUSIONS

1. In experimental insulin hypoglycemia it has been shown that, associated with the decline in blood sugar, there is an increase in the percentage of hemoglobin in the blood.

2. The concentration of the blood due to a loss of fluid is evident from an increase in hemoglobin percentage, an increase in the blood cell count, a decrease in the percentage of plasma, a reduction in the plasma volume and from certain physical changes in the blood associated with dehydration.

3. The experimental results fall under two main groups. In the first group the percentage of hemoglobin shows a gradual increase in value throughout the experimental period; in the second group the increase in hemoglobin develops rapidly. In the latter type of response a number of functional disturbances appear to be associated in the production of a profound physiological upset in which blood concentration seems to play an important part. We have designated the resulting condition *hyperinsulin dyshexia*.

4. Some of the practical bearings of our observations have been discussed and an attempt made to relate certain of them to the observations of others upon human subjects with insulin hypoglycemia. These observations, which emphasize the loss of much water from the body and the especial danger when insulin is given to those in a desiccated state, can readily be related to fluid loss from the blood.

We wish to thank Eli Lilly & Company for supplying the insulin and the anesthetic used in these experiments.

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OXYGEN CONSUMPTION IN MEN DURING SHORT EXPOSURES TO LOW BAROMETRIC PRESSURES

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While the influence of variations in barometric pressure, and the partial pressure of oxygen, on the gaseous exchange of the animal body has been frequently studied, it can not be maintained that our knowledge of the effects produced is at all complete. As early as 1873 Pflüger (14) claimed that the oxidative processes of the body are independent, within wide limits, of the oxygen supply. Later, in a study of themsleves, A. Loewy, J. Loewy and L. Zuntz (10) found that for one subject while at rest in a pneumatic cabinet at a barometric pressure of 448 mm. the gaseous metabolism did not materially change. The oxygen consumption at 758 mm. was 231 cc. and at 448 mm., 239 cc.; while the carbon dioxide output was 200 and 213 cc. respectively. But when they were in the mountains at an altitude of about 12,000 feet they showed definite increases in oxygen consumption of 6.3, 20.1 and 16.5 per cent respectively.

At an altitude of 15,000 feet, barometric pressure 443 mm., Durig and Zuntz (5) obtained an increase of as much as 15 per cent in the resting metabolism; but found no change at 10,000 feet and again in another expedition observed no change at 10,500 feet. Durig (6) and Fuchs and Deimter (7) likewise obtained increases at a barometric pressure of 443 mm. Durig in 1909 reviewed all data from the available literature of the various Alpine expeditions up to that date and found that in a majority of individuals the gaseous metabolism had been increased at altitudes that ranged from 6,600 to 15,000 feet (590 to 425 mm.).

Results opposed to those just cited have come from more recent investigations. Douglas, Haldane, Henderson and Schneider (4) found in Douglas, on Pike's Peak, altitude 14,110 feet, when at rest in bed, that the average gaseous exchange was 248 cc. of oxygen and 206 cc. of carbon dioxide and that in Oxford these averaged 237 cc. and 197 cc. respectively. Hasselbalch and Lindhard (8) by means of a pneumatic chamber found that one subject, who remained 18 days at a pressure ranging from 552 to 448 mm., had an average consumption of 226 cc. of oxygen and an average output of 176 cc. of carbon dioxide; while at the normal barometric pressure the absorption

of oxygen was 219 cc. and the excretion of carbon dioxide 173 cc. In another experiment of 6 days', and in several of shorter duration the gaseous exchange was affected still less. They concluded, therefore, that variations in barometric pressure had no influence on the consumption of oxygen. It should be noted, however, that in persons who experienced symptoms of mountain sickness the absorption of oxygen was clearly above normal during the period of illness and that it returned to the normal rate with improved health. Schneider (15) in a study on Pike's Peak found that 2 of 3 men experienced no change, or at most only a slight increase, in the gaseous metabolism; while during the first 48 hours at the high altitude the third experienced an increase that averaged 17.6 per cent for oxygen and 14.2 per cent for carbon dioxide. This was followed by a return to his normal rate of metabolism.

In the Andes Expedition (1) of 1922 a few determinations of the basal metabolism were made on 6 men. Of these 3 showed a slight decrease and 3 an increase.

Krogh (9) is of the opinion that the physiologic conditions during mountain expeditions become too complicated to allow conclusions to be drawn with regard to the influence upon metabolism of the oxygen pressure when taken by itself. He cites the investigations of Thunberg (16) upon animals to demonstrate that the oxidations taking place within the living body vary with the changes in the oxygen supply. The velocity of a chemical reaction, it is pointed out, depends upon the number of molecules able to take part in it and if the molecules of one of the substances become fewer in number the velocity of the reaction must decline. He came to the conclusion that the experimental results from the various investigations upon the effects of variations in the oxygen supply upon the respiratory exchange in man, and in warm-blooded and cold-blooded animals, in their entirety show that the oxygen pressure is practically the limiting factor for the oxidations of the body; but that the oxygen pressure is ordinarily so regulated as to be just sufficient for the body need. He concluded that at all pressures of the atmosphere higher than 410 mm. the average oxygen tension in the capillaries remains practically the same, because of adequate compensatory changes, and that there is no reason why with a constant supply of oxygen, the absorption should not also remain constant. He further concluded that a diminution of the oxygen supply to the tissues, which will take place when the oxygen pressure in the inspired air falls below about 83 mm. (atmospheric pressure 410 mm.), will result in a decrease in the rate of oxidation.

We have recently made a study of the gaseous metabolism of men and women during short exposures to a low barometric pressure in a low pressure chamber (13). Our results support in general the conclusions reached by Krogh. These indicate that the decrease in the rate of oxidation is only

temporary and that individuals are not all affected to the same extent and that in any one person the effect is not necessarily the same on different days.

Others have made their observations on men under relatively moderate changes in pressure and have made infrequent determinations of the rate of gaseous exchange. It has been our purpose to subject men to a pressure low enough to clearly stimulate the heart and respiratory mechanism and to induce other changes such as disturbed acid-base equilibrium and concentration of the blood. Hence we have employed pressures that are certain to bring on the symptoms of "mountain sickness" in the unacclimatized if held under the reduced pressure long enough. In most of our experiments the time allowed was too short to cause illness and even in our longer experiments we tried to terminate the run before the subject was really ill. By frequent determinations of the metabolism we have been able to demonstrate changes that may be expressed in the form of a curve.

PLAN AND METHODS. There are 4 series of experiments. In each of these the barometric pressure was reduced at the rate of approximately 21 mm. a minute (equivalent to ascending 1000 feet) until the desired pressure was obtained, after which it was maintained at practically a constant level. Insofar as was possible all experiments were made during the late forenoon so as to eliminate the effects of breakfast and of any physical exercise taken in coming to the laboratory. Physical exertion was reduced to a minimum for an hour to an hour and a half before the experiment and, immediately preceding the first collection of expired air, the subject was seated as quietly as possible for a period of about 20 minutes. In all experiments, except those of the fourth series, the subject remained at rest in the seated posture through the entire period of the run.

The Douglas bag was used for the collection of expired air and all analyses of the air were made with the Haldane apparatus. The normal rate of metabolism was determined just before the pressure was reduced. Record was also made of the oxygen content of the air in the chamber, the temperature and the cooling power of air as determined by the kata-thermometer.

The plan of the experiments was as follows: Series 1. The pressure was lowered to 380 mm., approximately equal to an altitude of 18,000 feet, and held there for a period of from 30 to 60 minutes. The rate of metabolism was first determined during the period of decompression as the pressure was being lowered from 560 to 380 mm., a second determination was made immediately after 380 mm. was reached and determinations 3 and 4 at regular intervals that varied somewhat in different experiments.

Series 2. The pressure was lowered by stages or steps, first to 515, then 425, 350 and 290 mm., approximately comparable to 10,000, 15,000, 20,000 and 25,000 feet in altitude. The pressure was maintained at each

level for 10 minutes before a 10-minute period of collection of the expired air was begun. The interval of 10 minutes was allowed for the subject to come into some degree of respiratory equilibrium.

Series 3. The pressure was lowered to 350 mm., approximately equivalent to an altitude of 20,000 feet, and maintained during a period allowed for physiological adjustment, for the collection of expired air for the metabolism determination and the collection of 2 samples of alveolar air and one of venous blood from a vein of the arm.

Series 4. Pressure was reduced to 380 mm. and maintained for from 4 to 6 hours, during which time metabolism determinations were made as follows: 10 minutes, 30 minutes, 60 minutes, and hourly thereafter.

In attempting to determine the influence of the anoxemia, of such barometric pressures as we have employed, on the gaseous metabolism of the body, it is necessary to consider the intake of oxygen separately from the output of carbon dioxide. In the short-time exposures there is always some washing out of carbon dioxide because of the response of the respiratory center to the lack in oxygen. This, of course, alters the respiratory quotient and gives a seeming change in the character of the metabolism. For this reason the consumption of oxygen gives the best index of the amount of oxidation that occurs in the body during the early stages of anoxemia.

If the question as to whether or not variations in oxygen pressure influence the use of oxygen by the body tissues is to be correctly answered it becomes necessary to correct all data for any increase or decrease in oxygen consumption that may result from changes in muscular contraction. Usually exposure to a decreased oxygen pressure causes an acceleration of the heart and an augmentation in the minute-volume of breathing which necessitate some increase in the demand for oxygen by the muscles of the heart and the respiratory apparatus. It has been estimated by Loewy and Schrotter (11) that for a man at rest the metabolism of the heart amounts to about 4 per cent, and with increased activity to as much as 15 per cent, of the total for the whole body. The metabolism for the respiratory movements of man was determined by Speck (9) to amount to from 8 to 10 cc. of oxygen per liter of air respired. Loewy (9) later estimated the oxygen consumption at about 5 cc. per liter of ventilation and Bornstein and Gartzen (2) at 5.6 cc. of oxygen.

In order to interpret our findings we have, therefore, ventured to make a correction for increased breathing. But since the amount of oxygen consumed for each heart beat is unknown, no correction has been made for the change in heart rate. For the changes in breathing a correction of 5.5 cc. in the oxygen consumption has been allowed for each liter of change in the air respired.

Series 1. We have 16 experiments with 7 subjects in which four collections of exhaled air were made in 10 minute periods; the first as the

atmospheric pressure was being lowered from 560 to 380 mm., the second immediately after 380 mm. was reached, and the third and fourth, separated

TABLE 1

PERSON	DATE	BAROMETER	COLLECTION OF 10 MINUTES	BREATHS PER MINUTE	MINUTE VOLUME	REDUCED TO 0° AND 760 MM.		R.Q.	PULSE RATE
						O_2	CO_2		
		mm.			*liters*	*cc.*	*cc.*		
K. O. N.	2/14	760	N	12	5.80	200	180	0.90	79
		560–380	1	13	6.87	215	184	0.86	82
		380	2	9	6.74	219	170	0.78	82
		380	3	12	5.95	205	172	0.84	81
		380	4	13	6.28	201	171	0.85	91
E. C. S.	2/17	760	N	10	6.20	237	174	0.73	72
		560–380	1	11	7.53	240	174	0.73	78
		380	2	14	9.63	231	193	0.84	84
		380	3	15	9.12	240	172	0.72	82
		380	4	15	8.95	241	176	0.73	85
N. P.	5/18	760	N	10	6.39	264	225	0.85	71
		560–380	1	12	7.58	249	242	0.97	76
		380	2	12	7.84	224	204	0.91	72
		380	3	13	7.85	228	205	0.90	75
		380	4	11	8.94	255	218	0.86	76
H. H. E.		760	N	10	6.37	269	203	0.75	79
		560–380	1	11	5.98	243	206	0.85	85
		380	2	12	8.02	216	202	0.94	84
		380	3	12	9.41	249	220	0.88	79
		380	4	12	8.98	272	206	0.76	79
G. C.	6/19	760	N	15	6.62	209	181	0.87	94
		380	1	18	7.41	210	184	0.88	101
		380	2	16	7.05	203	173	0.85	113
		380	3	16	6.72	213	176	0.83	108
		380	4	16	7.39	228	184	0.81	109
C. W.	5/5	760	N	9	5.38	218	173	0.79	73
		380	1	12	5.78	192	171	0.89	83
		380	2	12	6.88	196	172	0.88	89
D. T.	5/8	760	N	9	5.2	180	145	0.81	71
		560–380	1	11	5.7	193	160	0.83	82
		380	2	14	7.5	204	170	0.83	84
		380	3	14	7.7	195	165	0.85	79
		380	4	13	7.6	176	151	0.86	75

from the preceding collections by 5 minute periods. The data for a typical experiment on each of the 7 subjects will be found in table 1. The

figures there given for oxygen consumption have not been corrected for the increased muscular effort of stimulated respiration and circulation.

The experiment recorded in table 1 for K. O. N. was the first of four made on him and is on the whole typical of the lot. This subject never had an appreciable increase in the minute-volume of breathing, consequently no correction has been applied in his case. His data for oxygen consumption averaged as follows: normal, 229.5 cc; last 10 minutes of the period of decompression, 223 cc.; first collection at 380 mm., 226 cc.; second at 380 mm. 222 cc.; and the third at 380 mm., 231 cc. In this man the oxgyen consumption was practically unaffected by the lowered barometric pressure. There was a slight tendency toward reduction in the intake of oxygen at the time of the first collection of respired air, but the absorption was again normal during the last collection.

In two experiments on E. C. S., the first of which is given in table 1, a decrease in oxygen consumption was indicated when the correction for increased breathing was applied. In these experiments the normal metabolism showed intakes of oxygen of 237 and 240 cc. respectively; the last determinations at 380 mm., when corrected, were 226 and 216 cc., reductions of 4.6 and 10 per cent respectively. The decrease in consumption of oxygen was not present during the period of decompression, but was clearly present in each experiment for the 3 determinations made at 380 mm.

There were 3 experiments with N. P., the first of which is recorded in table 1. His normal intake of oxygen averaged 266 cc. The averages for the corrected data were as follows: during decompression 230 cc. (uncorrected 238 cc.), first collection at 380 mm. 223 cc. (uncorrected 234 cc.),second 241 cc. (uncorrected 250 cc.), and third 247 cc. (uncorrected 259 cc.). In each experiment the consumption of oxygen decreased during decompression, and was reduced still more during the next ten minutes so that there was a total average decrease of 16.1 per cent. There was a tendency to recover in the following 30 minutes, but the consumption still remained approximately 7 per cent below normal at the time of the last determination.

In 4 experiments with D.T. there were two that were almost alike, the data of one are given in table 1. The normal intake of oxygen in each experiment was 180 cc. and the corrected intake for the last determination of each was 163 cc., thus making a reduction of 9.5 per cent. In the other two experiments with this subject a large increase in oxygen consumption occurred that can not be accounted for by any of our data. In one instance the normal intake was 196 cc. During decompression it fell to 131; but later rose to 242 cc. (uncorrected 275 cc.), 284 cc. (uncorrected 337 cc.) and 258 cc. (uncorrected 312 cc.). In the other experiment the normal intake was 146 cc., during decompression 117 cc. (uncorrected 151 cc.) and later 213 cc. (uncorrected 254 cc.). In these last two experiments the minute-volume of breathing increased from about 5.5 L. to 15 L. That

the absorption of oxygen of the first two experiments represents the normal response of this subject is evidenced in other data to be given later (see tables 2 and 4).

Only a single experiment was made on each of the subjects, H. H. F., C. W., G. C., the results of which are given in table 1. H. H. F. showed a decrease in oxygen consumption which began during decompression, but was more pronounced in the first 10 minutes spent at 380 mm., later some recovery occurred, nevertheless the absorption was still approximately 4 per cent below normal at the time of the last determination. C. W., who did not tolerate the experience of anoxemia well, showed a decrease in oxygen intake of approximately 14 per cent. G. C. in the first 3 determinations of oxygen intake showed no change, but in the last gave evidence of an increased consumption of about 7 per cent.

From this set of observations it appears that an anoxemia, caused by reducing the barometric pressure at the rate of 21 mm. per minute down to 380 mm., may in some persons decrease, at least temporarily, the oxidative processes in the body. Among 7 subjects there were 3 who first experienced the decrease during the period of decompression, while in 2 others it occurred later. The decrease in oxygen consumption when a correction was made for the effort of increased breathing ranged between 4.6 and 23 per cent. Usually a tendency to return to the normal rate of oxidation was in evidence after a period of 20 to 30 minutes had been spent at the low atmospheric pressure. It also appears that sometimes the rate of metabolism may be increased without apparent cause, as was the case on two occasions in one subject who normally experienced a reduced intake of oxygen under the same barometric pressure. A pressure of 380 mm. was entirely without effect on one individual.

Series 2. The effects of decompression by stages or steps have been followed to determine where the oxidative process is first affected by the decrease in available oxygen.

There are 8 experiments in this series in which the barometric pressure was lowered at the usual rate, first to between 528 and 535 mm. (approximately equal to an altitude of 10,000 feet) next to between 443 and 450 mm. (15,000 feet), then 371–378 mm. (20,000 feet), and in 3 cases finally to 312 mm. (25,000 feet). The results for 6 of the cases are given in table 2. In five of the 8 cases a reduction occurred in the consumption of oxygen, while in the other 3, who were not carried beyond 378 mm., there was no change.

R. W. C. showed no change in oxygen consumption at 530 mm. but the corrected data indicate a decrease of 7.4 per cent at 445 mm., of 14 per cent at 373 mm., and 17.5 per cent at 312 mm. For D.T. it may be concluded that, while the consumption of oxygen was somewhat above normal at 535 mm., there really was no change until the 378 mm. level was reached, where a

decrease of 8.9 per cent was present; while at 317 mm. a decrease of 17 per cent was indicated. In two experiments reported in the previous

TABLE 2

	SEA LEVEL	10,000 FEET	15,000 FEET	20,000 FEET	25,000 FEET
R. W. C.					
Minute volume in liters	6.8	8.0	7.4	8.9	11.9
Oxygen consumption in cc.	256	261	240	232	239
Carbon dioxide output in cc.	221	213	200	204	216
Respiratory quotient	0.859	0.816	0.835	0.881	0.903
D. T.					
Minute volume in liters	5.4	6.4	6.6	8.2	10.1
Oxygen consumption in cc.	180	204	189	179	175
Carbon dioxide output in cc.	156	168	167	164	163
Respiratory quotient	0.867	0.825	0.881	0.915	0.931
L. T.					
Minute volume in liters	7.5	9.8	9.8	8.8	
Oxygen consumption in cc.	284	298	273	266	
Carbon dioxide output in cc.	266	279	267	237	
Respiratory quotient	0.934	0.936	0.976	0.890	
J. K. S.					
Minute volume in liters	5.2	6.9	6.5	8.8	
Oxygen consumption in cc.	195	230	209	215	
Carbon dioxide output in cc.	166	185	174	186	
Respiratory quotient	0.850	0.802	0.834	0.863	
N. P.					
Minute volume in liters	6.3	5.9	6.2	7.2	
Oxygen consumption in cc.	231	216	211	218	
Carbon dioxide output in cc.	182	168	173	201	
Respiratory quotient	0.788	0.777	0.819	0.922	
H. H. F.					
Minute volume in liters	6.1	5.9	7.6	14.6	14.9
Oxygen consumption in cc.	258	250	251	299	284
Carbon dioxide output in cc.	186	185	218	288	280
Respiratory quotient	0.740	0.721	0.869	0.963	0.986

series this subject gave practically the same amount of decrease at 380 mm. as occurred in this experiment.

In one other case, H. H. F., the pressure was lowered by steps to 317 mm. His consumption of oxygen when corrected for the increased respiration

at the several levels, beginning with sea level, was 258, 250, 243, 252 and 236 cc. respectively. While a slight decrease in the use of oxygen is indicated at all levels the reduction was only pronounced and, therefore, definite at the last level, 317 mm., at which time it was 8.5 per cent below normal.

Case L.T. gave no change at 528 mm., but showed a reduction at both 443 and 371 mm. of about 8.5 per cent. N. P. was the only subject who showed a decrease in the oxygen intake as early as 530 mm., when it was 6.5 per cent below normal. At 450 mm. the decrease was 8.7 per cent and at 375 mm., 7.8 per cent. The data for J. K. S. that appear in table 2 are typical for the three subjects who showed no definite change at any of the three barometric pressures to which they were exposed.

This series of experiments brings out clearly the fact that individuals are not affected by a reduction in atmopsheric pressure in equal degree. Of the 8 cases one showed a reduction in the oxidations of the body at a barometric pressure of 530 mm., partial pressure of oxygen 110 mm.; two cases at 443 mm., partial pressure 92 mm.; one at 378 mm., partial pressure 79 mm.; another at 317 mm., partial pressure 66.; while 3 men were unaffected at 378 mm., which was the lowest pressure to which they were subjected.

Series 3. If a reduction in the oxidations of the body actually occurs it should be possible to prove by other means that the tissues reduce their intake. For this purpose we have attempted to determine the amount of oxygen that is withdrawn from 100 cc. of blood as it flows through the hand.

In this series of experiments the oxygen content of the alveolar air, the arterial and venous blood will be considered, as well as the changes in the oxidative processes. The data for all the experiments, 14 on 6 subjects, are given in table 3. Among the subjects are 3 who appear in one or more of our other series.

It should be noted that in these experiments the pressure was gradually lowered, in 20 minutes, to 350 mm. and then maintained as steadily as possible at this level. A period of at least 10 minutes was spent before making the first observation.

R. W. C., in 3 experiments, had an average consumption of oxygen of 244 cc. at 760 mm. and 223 cc. (236 cc. uncorrected) at 358 mm., an average reduction of 8.6 per cent. The minute volume of breathing averaged 6.1 L. at 760 mm. and 8.4 L. at 358 mm. C. R. J., in 5 experiments, gave an average absorption of oxygen of 297 cc. at 760 mm. and of 268 cc. (uncorrected 272 cc.) at 350 mm., an average decrease of 9.8 per cent. E. C. S., in 2 experiments, had an average intake of oxygen of 238 cc. at 760 mm. and of 217 cc. (uncorrected 229 cc.) at 350 mm., a decrease in oxidation of 8.8 per cent. It appears, therefore, that R. W. C., C. R. J. and E. C. S. reduced their consumption of oxygen in almost equal degree when under a partial oxgyen pressure of 73 mm.

TABLE 3

SUBJECT	DATE	BAROMETER	MINUTE VOLUME	RESPIRATORY EXCHANGE REDUCED TO 0° AND 760 MM.		R.Q.	ALVEOLAR AIR		ARTERIAL BLOOD SATURATION	VENOUS BLOOD SATURATION	DIFFERENCE BETWEEN ARTERIAL AND VENOUS BLOOD	HEMOGLOBIN	PULSE RATE
				Oxygen consumption	Output of carbon dioxide		Oxygen	Carbon dioxide					
			liters	*cc.*	*cc.*		*mm. Hg*	*mm. Hg*	*per cent*	*per cent*	*cc.*	*per cent*	
R. W. C.	1/25	760	6.4	243	224	0.922	103.3	37.5	97	69	5.2	100	74
		350	7.2	215	228	0.943	37.5	29.3	77	55	4.8	119	88
	4/17	760	5.9	247	202	0.818	103.2	39.6	97	61	7.0	108	72
		350	9.2	255	258	1.012	32.1	32.9	67	39	5.9	114	78
	4/24	760	6.0	243	199	0.820	101.4	37.2	97	62	7.3	114	74
		350	8.8	238	215	0.904	30.6	33.2	64	40	5.5	123	84
C. R. J.	2/14	760	7.2	263	247	0.938	100.9	39.5	97	81	2.6	90	71
		350	7.9	248	233	0.939	33.4	32.4	69	49	3.8	98	96
	2/20	760	8.5	325	296	0.912	101.0	41.0	97	51	8.5	100	78
		350	8.8	253	251	0.993	32.3	33.7	67	43	4.7	105	98
	3/23	760	7.3	294	240	0.817	97.2	40.0	96	64	5.8	95	76
		350	8.6	292	250	0.858	33.1	31.9	69	45	4.7	106	99
	4/6	760	8.2	326	275	0.844	103.4	39.4	97	58	7.3	100	74
		350	9.0	274	264	0.964	35.5	31.4	72	45	4.8	96	91
	5/1	760	7.7	279	242	0.868	103.6	39.8	97	61	6.7	100	79
		350	8.6	292	261	1.036	36.8	34.5	66	47	4.0	109	102
E. C. S.	1/5	760	5.5	223			101.1	39.3	96	63	6.8	110	72
		350	7.1	211			30.8	31.7	64	28	8.3	124	94
	3/2	760	5.2	253	194	0.767	100.2	40.4	96	59	7.0	104	72
		350	7.8	246	239	0.962	31.6	34.4	63	32	6.7	115	94
H. H. F.	3/28	760	7.4	285	213	0.748	95.6	42.6	95	72	4.1	95	90
		350	7.9	273	256	0.938	21.0	41.7	38	26	2.2	99	105
	4/11	760	7.8	250	234	0.935	91.2	42.9	96	73	4.9	114	86
		350	10.1	263	269	1.022	29.6	35.2	60	50	2.2	118	86
L. H. C.	5/8	760	9.1	250	264	1.035	101.2	32.9	97	61	6.6	100	82
		350	9.8	256	246	0.961	41.2	28.1	81	50	5.8	102	89
J. C. B.	6/2	760	8.5	322	271	0.841	105.0	37.3	97	61	7.3	110	67
		350	8.3	236	234	0.992	29.0	34.2	60	42	3.9	115	100

H. H. F. served in 2 experiments in one of which the oxygen intake was not altered, while in the other it was decreased 5.4 per cent. The oxygen intake of L. H. B. was unaffected by the reduced barometric pressure. J. C. B., in one experiment, showed a decrease of 26.6 per cent in his oxidative processes.

Our study of the oxygen content of the blood of the arm indicates that the consumption of oxygen by the tissues was decreased while the men were under a low barometric pressure. For the purpose of estimating the amount of oxygen delivered to the tissues by 100 cc. of blood we determined the arterial blood content of oxygen by an indirect procedure and analyzed a sample of the venous blood, drawn from an arm vein, by the Van Slyke method, for its content of oxygen. The oxygen content of the arterial blood was estimated from determinations of the alveolar oxygen pressure and the hemoglobin content of the blood. After deducting 5 mm. from the alveolar oxygen pressure, as suggested by Barcroft, the percentage of saturation of the arterial blood with oxygen was estimated by the use of a generalized blood oxyhemoglobin dissociation curve. Having, therefore, an estimated value of the degree of saturation of the arterial blood we next calculated the oxygen content in cubic centimeters from the known hemoglobin content. Our determination of hemoglobin was made by methods in which the standard value was 18.5 cc. of oxygen per 100 cc. of blood. For a part of the hemoglobin determinations we used the method of Cohen and Smith (3) and for the remainder that of Newcomer (12).

In general our data indicate that at a barometric pressure of 760 mm. the arterial blood is 97 per cent and the venous blood 62 per cent saturated with oxygen, and that at 350 mm. the arterial blood is 62 per cent and the venous blood 42 per cent saturated. The hemoglobin content averaged 103 per cent at 760 mm. and 110 per cent at 350 mm. From these data the oxygen content of 100 cc. of blood is found to be, at 760 mm. 18.5 cc. for the arterial blood and 11.8 cc. for the venous blood, a difference of 6.7 cc.; and at 350 mm., 12.6 cc. for the arterial blood and 8.5 cc. for the venous blood, a difference of 4.1 cc. Hence the withdrawal of oxygen from the blood was approximately 39 per cent less at a barometric pressure of 350 mm. than at 760 mm.

An examination of the individual cases brings out some irregularities; but, on the whole, the data are concordant. In 3 experiments at 760 mm., the arterial blood of R. W. C. was 97 per cent saturated; while the venous blood was 69, 61 and 62 (average 64) per cent saturated. The experiments on him extended over a period of 3 months, during which time his hemoglobin increased 14 per cent. All these experiments showed that less oxygen was withdrawn from 100 cc. of blood as it circulated through the arm when he was under the low barometric pressure of 350 mm. than when under a normal barometric pressure. At the normal barometric pressure

the blood yielded to the tissues 5.2, 7.0 and 7.3 (average 6.5) cc., while at 350 mm. it gave 4.8, 5.9 and 5.5 (average 5.4) cc.

C. R. J., in 5 experiments, had an average saturation of the arterial blood at 760 mm. of 96.8 per cent and of the venous blood of 63 per cent; while at 350 mm. these were 68.6 and 45.8 respectively. In one of the experiments less oxygen was taken up by the tissues from the blood when the subject was under the normal barometric pressure than when under the reduced pressure, the amounts used at the two pressure were 2.6 and 3.8 cc. respectively. In the remainder of the experiments the reverse condition obtained, so that the average withdrawal of oxygen from 100 cc. of blood was 6.2 cc. at 760 mm. and 4.4 cc. at 350 mm.

The two experiments on E. C. S. do not give concordant results. In the first 6.8 cc. of oxygen were withdrawn from 100 cc. of blood under normal conditions and 8.3 cc. at the low barometric pressure. In the second experiment the withdrawal was 7 cc. at 760 mm. and 6.7 cc. at 350 mm., a difference too small to be significant.

In the two experiments on H. H. F. the withdrawal of oxygen from the blood was 4.1 and 4.9 cc. per 100 cc. of blood under the normal condition and 2.2 cc. in each exposure to 350 mm. It should be recalled that in the first experiment a reduction in the total body consumption of oxygen occurred, while in the second there was no change. The discrepancy is unaccounted for in our data. A similar case is that of L. H. B. In him the total consumption of oxygen was unaffected by the lowering of barometric pressure. In spite of this the withdrawal of oxygen from 100 cc. of blood in the hand was slightly less when he was under the reduced pressure. He withdrew 6.6 cc. of oxygen at the normal barometric pressure and only 5.8 cc. at 350 mm.

The largest reduction in the total absorption of oxygen by the body due to the lowered barometric pressure was obtained in J. C. B., who showed at 530 mm. a reduction of 26.6 per cent. It is interesting, therefore, to find that he also markedly changed the withdrawal of oxygen from the blood of the arm. At the normal barometric pressure the blood lost 7.3 cc. per 100 cc. as it flowed through the arm; while at a pressure of 350 mm. the loss was only 3.9 cc. Also in one experiment, the second, on C. R. J. the reduction in the total bodily absorption of oxygen was unusually great, that is 22 per cent; and with this was associated a large difference in the withdrawal by the arm at the two barometric pressures. This man withdrew 8.5 cc. of oxygen at 760 mm. and 4.7 cc. at 350 mm., a difference of 3.8. In one other case, that of J. C. B., we obtained a difference of 3.4 cc. per 100 cc.

This series of experiments adds further support to the evidence of our first two series which showed that the total consumption of oxygen by the body may be reduced by a lowered partial pressure of oxygen. The

experiments also give proof that the gaseous metabolism in the tissues of the body appendages is at the same time reduced. That the reduction in oxidation indicated by the blood study is real, and not just apparent, would be proved if it could be shown that the blood flow through the limbs of the body is not increased at the same time. We have experiments on the rate of blood flow through the arm that will be published in another paper which demonstrate that the flow is not increased, but rather that it is decreased. Therefore, it seems clear that, for a time at least, the oxidation processes in the arm may be reduced when the partial pressure of atmospheric oxygen is lowered to 80 mm. The experiments in series 2 indicate that in an occasional person the metabolism may be influenced before the pressure of oxygen is lowered to 80 mm., in some cases a change was observed at about 100 mm. of oxygen.

Series 4. In this series of experiments we believe we have obtained evidence that proves the reduction in the oxidative processes of the body to be only temporary. In some individuals the metabolism soon returns to normal; while in others, as discomfort and evidences of "mountain sickness" appear, it rises above normal. Studies made by Schneider on Pike's Peak brought out the fact that during the first days of residence at a high altitude the rate of metabolism may be higher than is normal for low altitudes. Our experiments suggest that the temporary increase observed by Schneider may have been associated with the disturbances of maladjustment or delayed adjustment to the new environmental condition.

In this series of experiments our subjects were at a barometric pressure of approximately 400 mm. for periods of from 3.5 to 8 hours, during which time the noon meal was omitted. Recorded observations of the sensations of the subjects are of interest. D .T. at first felt natural and comfortable, then between the first and second hour felt faint. There were peculiar skin sensations, twitching of the muscles of the forehead and a general feeling of weakness. This continued for more than an hour, then a short sleep with the head down on the arms restored her. No other symptoms were noticed while in the low pressure chamber; however, for a time after the experiment a feeling of weakness and lack of coördination of movements was reported. Later a headache developed which became more severe during the evening. L. T. noticed a narrowing of his field of vision early in the experiment and felt heart palpitation. He attempted to read, but found it difficult to do so. He seemed unable to get the meaning of what he read and reread almost every paragraph. He experienced effort in focusing the eyes on the print. Some headache occurred and abdominal pain. The experiment was terminated because discomfort was increasing. After the experiment the headache was very severe, he was pale, suffered from nausea and vomiting, and was unable to eat supper. He had fully recovered by the following morning. In one experiment R. W. C. felt natural for more

than an hour then, as his record reads, "I began to feel irritable and excited and realized at the time that I was talking much and without any particular purpose or sense, but had no inclination to stop." He was sleepy by spells, and slept between several of the metabolism determinations. He was pale during the middle of the experiment, but the color improved as time passed. A slight headache came on at the close of the experiment and persisted after the experiment into the night. For half an hour or more after the experiment he felt unsteady. In the second experiment on R.W.C. the effects during and after exposure to the low pressure were less noticeable.

The data for this series are given in table 4. The variations in oxygen intake were less for D. T. than for the other subjects. The actual determinations of the amount of oxygen consumed per minute are recorded in the table. After making the correction for increased breathing the following were found; normal, 186 cc.; at a barometric pressure of 398 mm. in the interval from the 10th to 20th minutes, 192 cc.; between the 25th and 35th minutes, 169 cc.; in 1.2 hours, 194 cc.; in 2.2 hours, in 182 cc.; in 3.2 hours, 188 cc.; and at the end of 4 hours, 191 cc. The decrease in oxygen consumption at the end of 30 minutes amounted to 9.1 per cent of the normal. This reduction is approximately the same as she had in 2 experiments in series 1 and 1 in series 2, in which the decrease in oxidation was 9.5, 9.5 and 8.9 per cent respectively. In this long exposure to a low barometric pressure the metabolism was back to normal by the end of the first hour. There was no further change during the next three hours.

Subject L. T. had a more uncomfortable experience than D. T., with very severe after-effects. It is interesting, therefore, to find in his case that the rate of metabolism was higher than normal at the end of the stay. His normal consumption of oxygen was 280 cc. At a barometric pressure of 411 mm. the amount consumed was, after a stay of 10 minutes, 243 cc., a reduction of 13.2 per cent; after a stay of 30 minutes the consumption had returned somewhat toward normal, and was then 266 cc.; at 1.2 hours it was 258 cc.; in 2.2 hours, 260 cc.; and in 3.2 hours, 298 cc. For a period of more than two hours his metabolism was subnormal; but at the last, when he was clearly showing distress, the rate had not only returned to but was approximately 6 per cent above normal.

R. W. C., in experiment 1, had a normal consumption of 229 cc. of oxygen. Under a barometric pressure of 405 mm. his intake of oxygen dropped in 10 minutes to 220 cc., and in 30 minutes to 199 cc., a fall of 13.1 per cent. From then on there was a gradual return to normal, in 1.5 hours the consumption was 211 cc.; in 2.5 hours, 225 cc.; in 3.3 hours, 231 cc.; in 4.2 hours, 221 cc. At the very last, at the end of 5 hours, the intake, which was 281 cc. per minute, was approximately 23 per cent above normal.

In the second experiment on R. W. C., which lasted almost 8 hours, the metabolism was 15 per cent below normal for awhile. It then gradually

rose until at 5.5 hours it was 15.7 per cent above normal. Later observations showed it still about 4.5 per cent above normal. Very likely, had the experiment been continued longer, the metabolism would have remained high until acclimatization changes were well started.

TABLE 4

D. T.

	NORMAL TIME	10 MINUTES	25 MINUTES	1.2 HOURS	2.2 HOURS	3.2 HOURS	4 HOURS
Minute volume in liters	6.3	8.2	7.5	10.0	9.2	9.7	9.2
Oxygen consumption in cc.	186	192	176	214	197	207	207
Carbon dioxide output in cc.	156	170	149	189	167	167	152
Respiratory quotient	0.841	0.888	0.847	0.882	0.849	0.806	0.733

L. T.

	NORMAL TIME	10 MINUTES	25 MINUTES	1.2 HOURS	2.2 HOURS	3.2 HOURS
Minute volume in liters	5.5	8.4	9.2	8.0	7.5	8.7
Oxygen consumption in cc.	280	259	286	272	272	316
Carbon dioxide output in cc.	231	244	260	218	200	226
Respiratory quotient	0.826	0.942	0.907	0.800	0.736	0.717

R. W. C., I

	NORMAL TIME	10 MINUTES	30 MINUTES	1.5 HOURS	2.5 HOURS	3.3 HOURS	4.2 HOURS	5 HOURS
Minute volume in liters	6.1	7.3	6.8	7.3	7.4	8.2	8.6	11.6
Oxygen consumption in cc.	229	227	203	218	232	243	235	311
Carbon dioxide output in cc.	165	191	162	164	161	179	178	234
Respiratory quotient	0.720	0.840	0.799	0.751	0.694	0.737	0.759	0.751

R. W. C., II

	NORMAL TIME	10 MINUTES	27 MINUTES	1.5 HOURS	4 HOURS	5.5 HOURS	6.5 HOURS	7.5 HOURS
Minute volume in liters	7.1	8.1	8.1	9.2	10.4	10.6	10.7	11.4
Oxygen consumption in cc.	248	216	226	254	256	306	278	282
Carbon dioxide output in cc.	209	206	182	185	200	214	212	197
Respiratory quotient	0.842	0.954	0.805	0.730	0.781	0.699	0.761	0.704

A tendency for the metabolism to return to normal after a period of subnormal oxidation was noted in some of the experiments in series 1. N. P. in 3 experiments showed the upward trend in his oxidative processes, but he had not fully returned to normal at the time the experiments were terminated. H. H. F. in a single experiment partly returned. G. C., while not giving the usual reduction in the oxidative processes, showed in the last determination in a stay at 380 mm. a metabolism that was about

7 per cent above normal. D. T. in two experiments, not recorded in table 1, after having a subnormal oxidation for a few minutes showed an excessive metabolism which in one experiment was 31 per cent and in the other 45 per cent above normal.

From this series of experiments we are led to believe that under less severe conditions the metabolism after a period of subnormal rate would return to normal and remain there, and that under a greater reduction in barometric pressure the changes of metabolism describe a double curve. There is first a period of subnormal metabolism, then follows a gradual increase in the rate that leads eventually into a period of excessive oxidation, and finally, if satisfactory acclimatization changes occur within the body, the rate again returns to normal.

SUMMARY

1. The consumption of oxygen during anoxemia, caused by lowering the barometric pressure in a low pressure chamber to between 410 and 310 mm. for from 30 to 60 minutes, was reduced by from 4.5 to 26 per cent in 76 per cent of 42 experiments on 12 persons.

2. A decrease in the rate of oxidation was obtained in one case at a barometric pressure of 530 mm., partial pressure of oxygen 110 mm.; in 2 cases at 443 mm., partial pressure 92 mm.; in 1 at 378 mm., partial pressure 79 mm.; and in 1 case not until 317 mm., partial pressure 66 mm. was reached.

3. A study of the blood indicates that the gaseous metabolism of the arm is reduced, at least temporarily, by a low barometric pressure. In general, it was found that at a barometric pressure of 760 mm. the arterial blood was 97 per cent and the venous blood 62 per cent saturated with oxygen; and that at 350 mm. the arterial blood was 62 per cent and the venous blood 24 per cent saturated. The hemoglobin content of the blood averaged 103 per cent at 760 mm. and 110 per cent at 350 mm. The average oxygen content of 100 cc. of blood at 760 mm. was 18.5 cc. for the arterial blood and 11.8 cc. for the venous blood, and at 350 mm. 12.6 cc. for the arterial blood and 8.5 cc. for the venous blood. There was an average of 6.7 cc. of oxygen withdrawn from 100 cc. of blood at 760 mm. and only 4.1 cc. at 350 mm., making a reduction of approximately 39 per cent in the amount of oxygen taken from the blood by the arm.

4. In prolonged exposures of 3.5 to 8 hours to a barometric pressure of approximately 400 mm. the oxidations of the body, after a period of reduced rate, tended to return to normal and in some cases to rise above normal. The excessive rate of metabolism appeared to be associated with the onset of symptoms of "mountain sickness."

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STUDIES ON THE RELATIVE PHYSIOLOGICAL VALUE OF SPECTRAL LIGHTS

V. The Alleged Influence of Light upon Respiration in the Frog[1]

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The relative sensitiveness of frogs to lights of different wave-length has been investigated by various methods such as phototropic response, the action current of the enucleated eye and the seizure of illuminated food. These methods have been often used by investigators interested in the comparative physiology of color vision. Babák (1) has employed a not so common method, namely, the relative effects on respiration of light and darkness. He observed the respiratory movements, counting them and estimating their depth, of decerebrate frogs at least five months after operation. The specific effects of different wave-lengths obtained by passing white light through dye solutions, as well as the differences between respiration in light and darkness are, according to Babák, very clear-cut and distinctive.

In dim light, Babák describes the movements as regular and the rate constant (112 to 116 per minute), lung respiration occurring but rarely. In bright light, the rate is a little slower (102 to 100 per minute), with inflation of the lungs occurring relatively more frequently. In darkness, respiration becomes gradually slower and eventually periodic, with pauses of as long as a minute at a time. Single lung respirations occur only when the intensity of the illumination is changed and when a signal bell, which was used to mark the time intervals, sounds. In red and green lights breathing is similar to that in darkness, that is to say, slow and periodic with long pauses. In violet light, breathing is regular but faster than in bright white light (116 to 120 per minute), the depth is increased and the animals show signs of excitement such as blinking the eyes and moving out of the beam of light.

[1] The results here reported are taken from the essay presented by Elizabeth E. Crofts for the Degree of Master of Science, Yale University, 1924.

[2] Aided by a grant from the Elizabeth Thompson Science Fund.

Rouse (2) made a similar study of the effects of light on the rate and depth of respiration in the pigeon. Flashes of white light were found to disturb the respiration, the reaction consisting principally of an immediate quickening and shallowing with occasional pauses and irregularities. When red, yellow, green and blue lights were tested, the pigeons responded to the light stimulus by a quickening in rate, the average maximum rate being attained in the second respiration, after which it constantly diminished toward the normal, which was almost reached by the tenth respiration. Rouse further demonstrated a relation between the color of the light and the amount of its effect in the order of decreasing effectiveness: blue, green, yellow, red, which he found to be the order of "preference" when the birds were given the choice between them. He did not find any direct relation between the amount of stimulus and the amount of reaction when different intensities of lights were employed.

Babák's results on the frog were so specific and constant that it was considered of interest to repeat the experiments using lights of accurately known wave-length composition as well as of known energy content, and further to investigate CO_2 production. The latter has also been done before, but under rather imperfectly controlled conditions.

Decerebrate male specimens of *Rana pipiens* were used not sooner than three weeks after operation. Babák used frogs which had been operated upon several months previously and it might seem that for this reason our results and his might not be directly comparable. However, our animals showed no change in the general nature of the responses as time elapsed. Two of them have been in a decerebrate condition for five months. It was hoped at first that graphic records could be made and considerable time and energy was expended in devising a satisfactory method to do this. However, it was found impossible to avoid mechanical stimulation from the writing system as well as from the clockwork kymograph which obscured the record, so that it could not be used as an index of the effect of the light stimulus alone. The respiratory rate was therefore determined by observing the animals. The frog was placed in a small glass dish in water two or three millimeters deep, in a room completely dark except for a dim red photographic light placed on one side of the animal for the purpose of rendering visible the respiratory movements. This illumination was employed throughout the experiments so that any effects its radiation may have had was a constant one. That the animals were somewhat sensitive even to this dim light was shown by the fact that they sometimes turned so as to face it during the course of the experiment. When this happened, the frog was moved back to the first position and a new series of determinations begun. It was comparatively easy to observe the pharyngeal movements, but those due to lung inflation presented more difficulty. The method finally used was to fasten a strip of paper to the flank of the animal so that it projected

above the back. The movements of the flank due to the inflations of the lungs were in this way made simultaneously visible with the pharyngeal movements. The time, determined by a stop watch, taken for 50 pharyngeal respirations was selected as the unit of comparison.

In order to secure radiations of known wave-lengths, white light from a 500-watt monoplane filament stereopticon Mazda C lamp was passed through a Hilger constant deviation spectrometer. The lamp was enclosed in a box painted white on the inside and blackened without, and a blackened tube of adjustable length connected the slit of the collimator and the aperture in the light box. The whole apparatus, with the exception of the telescope, was covered with heavy black cloth, so that stray light was reduced to a minimum. The slit of the collimator was kept at constant width (1.321 mm.). An asymmetric slit was placed in the telescope tube and adjusted according to the method described by Laurens and Hooker (3) so that 30 wave-lengths were allowed to pass when the wave-length drum of the spectrometer was set at wave-length 589.3 mm. Since in the prismatic

TABLE 1

POSITION OF DRUM	RANGE OF WAVE-LENGTHS	DISTANCE FROM LAMP TO COLLIMATOR
$m\mu$		*cm.*
620	602.0–638	28.5
580	566.5–595	20.5
520	510.5–530	11.5
470	463.0–477	2.5

spectrum the dispersion increases as the wave-length decreases, the band of light emitted by the slit was composed of more than 30 wave-lengths toward the red end, and of less than 30 wave-lengths toward the blue end of the spectrum. In most of the experiments the energy content of the bands of spectral light was kept constant by changing the distance of the source from the collimator slit with each change in wave-length composition of the bands. In other experiments the relative energy was taken as it exists in the spectrum by keeping the distance between the source of light and the collimator slit constant for the bands of light. There was no difference in the results of the two series. Table 1 gives the actual wave-lengths emerging from the telescope for each setting of the spectrometer and also the distance of the source of light from the collimator slit necessary to furnish lights of equal energy content.

The regions of the spectrum selected were sufficiently widely separated so that differences in response, if any, would be distinct and unmistakable. The temperature was noted at the beginning and end of each experiment; but the variation during a single experiment was so small that it was found unnecessary to make any correction for it.

The frog was placed in position facing the spectrometer at a distance of 30 cm. from it, and allowed to remain thus in darkness, or rather in the very weak illumination of the photographic red light, for about half an hour before any counts were taken. The animals were usually quiet, though on some days an animal was so restless that experiments on it were discontinued. Upon observation the respiratory movements were found to be very regular and to consist principally of pharyngeal movements, inflation of the lungs occurring only at intervals. Contrary to Babák's account, the respiration in the dark was not periodic. The following series of counts, selected from many, taken after the preliminary half-hour of quiet, will illustrate this.

TABLE 2

PHARYNGEAL	LUNG	TIME IN SECONDS	
Frog IV. Temperature 21°–21°C.			
50	2	48.6	
50	1	49.6	
50	1	48.2	
50	0	51.6	Greatest difference 3.4
Frog VII. Temperature 22°–22.5°C.			
50	0	33.2	
50	0	33.4	
50	0	35.0	
50	0	34.0	
50	0	34.8	
50	0	33.8	Greatest difference 1.8
Frog VI. Temperature 20°–20°C.			
50	1	31.0	
50	0	29.6	
50	0	29.6	
50	0	29.2	
50	1	30.2	Greatest difference 1.8

The observations on respiration in darkness and in white light will first be described. The white light was the general illumination of the room furnished by four 100-watt Mazda C lamps suspended from the ceiling. The table below gives a summary of the results obtained.

Frog IV showed a decrease in the time required to complete 50 pharyngeal movements immediately following upon the changes from light to darkness and darkness to light. In other words, either change accelerates the rate of respiration. In the former, the decrease amounted to 6.3 seconds, and in the latter, 5.2 seconds. After this initial increase, however, the rate gradually returned to its normal level, which was approximately the same in light or darkness. The greatest variation between the

average rate attained in darkness and in light was 1.6 seconds; which is within normal variation. Frogs VI and VII show the initial increase in rate immediately following the change in illumination in agreement with frog IV, the increase in rate caused by the change from darkness to light being the greater. Furthermore the average rate in darkness is somewhat slower than in light. This, however, is not always so for any animal, e.g., in frog VI, the rate in darkness as given in the first table is the same as the rate in light in the second table.

TABLE 3

	PHARYNGEAL	LUNG	TIME IN SECONDS	
Frog IV. Temperature 21°–21.5°C.				
Light	50	3	43.2	
	50	4	43.2	
	50	4	44.6	
	50	2	44.6	Average 43.9
Darkness:				
Immediately following change in illumination	50	3	37.6	Difference 6.3″
1′ after change in illumination	50	2	43.2	
5′ after change in illumination	50	0	42.6	
10′ after change in illumination	50	2	44.2	
20′ after change in illumination	50	0	45.4	Average 44.8″
Light:				
Immediately	50	3	39.6	Difference 5.2
3′ after	50	6	44.0	
6′ after	50	0	43.8	Average 43.9″
Frog VI. Temperature 20°–21°C.				
Light	50	0	29.2	
	50	1	30.2	
	50	2	30.8	
	50	2	29.8	Average 30″
Darkness:				
Immediately	50	4	27.6	Difference 2.4″
5′ after	50	2	33.0	
8′ after	50	3	36.4	
10′ after	50	2	36.4	Average 35.3″
Light:				
Immediately	50	4	26.0	Difference 10.4″
5′ after	50	0	30.4	
8′ after	50	0	40.4	Average 30.4″

TABLE 3—*Continued.*

	PHARYNGEAL	LUNG	TIME IN SECONDS	
Frog. VII. Temperature 20.5°–21°C.				
Light	50	0	26.4	
	50	0	27.0	
	50	0	27.0	Average 26.8″
Darkness:				
Immediately	50	3	24.4	Difference 2.4″
3′ after	50	0	33.8	
5′ after	50	0	34.2	
8′ after	50	0	34.2	Average 34.1″
Light:				
Immediately	50	0	27.6	Difference 6.5″
5′ after	50	0	25.2	
8′ after	50	0	26.2	Average 25.7′
Darkness:				
Immediately	50	0	25.2	Difference 0.5″
5′ after	50	0	34.8	Average 34.8″

From such experiments, of which these are only a few examples, it was concluded that the rate of respiration increases in response to a change in illumination, and that this increase is perhaps greater for the change from darkness to light than for the reverse. The effect, however, is a transitory one, the rate of respiration decreasing until it proceeds at a rate characteristic of the animal whether in light or darkness. It was further observed, in agreement with Babák, that there is a definite increase in the respiratory rate, and in the number of lung inflations, following noises or mechanical vibrations. As a matter of fact, the foregoing observations were made under rather disadvantageous conditions in this respect, for there was a great deal of pounding and noise due to construction work going on in the building, the effect of which was much more outstanding than any observed influence of light.

Notwithstanding the somewhat disappointing results from the point of view with which the experiments were undertaken, namely, to demonstrate a specific influence of light on respiration, it was considered of sufficient interest to investigate the relative effects of different wave-lengths. At this time we moved into a new building so that noise and mechanical virations were no longer a factor. The telescope of the spectrometer was provided with a simple shutter so that the light could be turned on without illuminating the frog. This avoided any effect which the mere sound of the electric switch might have had.

It has not been deemed advisable to give here more than a condensed

table illustrating the general nature of these results. The plus sign indicates that the average rate in light after some minutes is greater, by the

TABLE 4

MIDDLE POINT OF BAND OF LIGHT	FROG	DARKNESS TO LIGHT		LIGHT TO DARKNESS		DIFFERENCE BETWEEN AVERAGE RATES IN LIGHT AND DARKNESS
		Decrease in time required for first 50 respiratory movements	Decrease in time required for first 10 respiratory movements	Decrease in time required for first 50 respiratory movements	Decrease in time required for first 10 respiratory movements	
mμ		sec.	sec.	sec.	sec.	sec.
470	VI	4.4		2.0		+0.9
		3.8		1.3		+0.3
		2.0		3.1		+0.3
		3.5		0.9		+0.7
	XI	3.2		2.6		+0.2
	X	0.7		2.8		−0.1
520	VI	2.2	5.4	0.2	4.8	−0.5
		2.2	8.8	1.3	2.3	−2.9
		2.9	10.0	0.8	7.0	+0.4
	X	1.8	3.4	1.9	2.0	+0.6
		1.4	1.8	3.9	6.9	−1.6
		3.6	8.0		8.7	+0.3
		1.4	5.8	0.6	2.2	+0.2
580	VI	2.6		2.0		−0.4
		2.9		5.1		−0.7
		2.3	6.1	0.3	9.3	
		4.6	9.8		9.3	+0.5
	X	2.7		4.9		−0.4
		3.3	6.1		5.7	−0.4
	XII	1.8	5.4	3.2	5.4	0.0
620	VI	2.4	7.6	4.3	7.7	+0.6
		2.2	6.8	4.5	9.6	+0.7
	X	2.0	4.0	1.3	2.1	−2.1
		3.2	6.0	3.2	7.0	−1.0
	XII	1.0	2.8	0.6	4.4	−1.6
		2.2	4.6	4.5	9.5	+0.9

amount shown after it, than the average rate in darkness; and the minus sign, that the rate in light is less than that in darkness.

It was soon noted that the respiratory rate immediately following a change in illumination was much faster than it was later, so that the true magnitude of the immediate effect was masked by counting as many as the first fifty movements. In later experiments, therefore, the time taken for the first ten, as well as for the first fifty respirations was determined. The first figure was then multiplied by five, thus making it comparable with the other rates obtained.

It is possible to draw the following conclusions: The frog is sensitive to change in illumination, whether from light to darkness or darkness to light, and exhibits this sensitivity by an increase in the rate of the respiratory movements immediately following such a stimulus, but the amount of the increase bears no quantitative relation to the wave-length of the light. This increase is a transitory phenomenon and the rate finally assumed, after 2 minutes in the light, varies but little from that in darkness. The variation is well within the limit of experimental error and is not consistently higher or lower in the light. Respiratory movements due to inflation of the lungs show no constant change in response to changes in illumination. It was observed that they occur most frequently as a result of muscular movement, e.g., the struggle incident to placing the animal in position may produce flank movements equal to 50 per cent of the total number of respiratory movements, or to the mechanical stimulus induced by heavy hammering or the slamming of doors.

CO_2 production in light and darkness. Although the results reported above seemed to indicate no differential influence of light and darkness on respiration, it was thought that if there were any change in the metabolic processes of the animal, in light as contrasted with darkness, it would be most surely and accurately detected by a measurement of the CO_2 output. Moleschott (4) reports an increase in CO_2 production by frogs in the light of from $\frac{1}{12}$ to $\frac{1}{4}$ of that observed in darkness. The increase is less if the frog be blinded. Moreover different colored lights were found to have specific ratios of CO_2 production compared to that produced in darkness. His animals, however, were allowed to move about freely and he notes that they were considerably more active in the light. Since the muscles are the main CO_2 producing organs of the body, his conclusion that light had a direct effect upon the metabolism does not seem to be justified. Ewald (5) repeated the experiments using curarized frogs, and found an increase in CO_2 production in the light of only 1 per cent. He concludes that light has no influence upon CO_2 excretion in the frog when muscular movement is eliminated. See also Holmes (6) and Krogh (7).

It was considered of interest to repeat the observations on CO_2 production, improving upon previous investigations, first, by using animals in which muscular movement was prevented or reduced to a minimum without the use of drugs, and second, by employing a more sensitive method of measuring CO_2 in small amounts.

The apparatus used to determine the CO_2 output was essentially that devised by Osterhout (8). The time taken to change the reaction of the indicator solution (phenol red) from a pH of 7.3 to one of 7.0 was taken as the standard. The illumination necessary for matching the indicator solution with the standards was furnished by an electric light enclosed in a blackened box with the aperture (covered with daylight glass) placed immediately behind a frame containing the indicator tube and the standard. In this way it was found that the colors could be accurately compared though the rest of the room was in complete darkness.

The respiration chamber consisted of a box with glass windows inserted on two sides, paraffined within and with the joints shellaced to render it air tight. The frog was placed in the respiration chamber through an opening, then closed and sealed with Plasticene, and allowed to remain in darkness for fifteen minutes before the motor was started. The air was then pumped through the U-tube containing solid NaOH until the indicator solution reached a pH of 7.3. The stopcocks were then adjusted so that the air from the chamber passed directly into the indicator solution, without passing over the NaOH, a stop watch being started simultaneously and the time taken for the indicator solution to reach a pH of 7.0 was determined. The air was then again freed from CO_2 and another determination made. The motor used for circulating the air ran continusouly and at the same rate during a single series of observations so that any psychic effect the noise may have had upon the animal was a constant one.

The animal was inspected at intervals during the experiment to determine whether any alteration in position had taken place. It was discovered that the rate of production of CO_2 was quite constant if the animals were motionless, but muscular movement varied the rate tremendously. The following two series of separate and consecutive determinations in darkness may be contrasted in this respect.

When the rate of CO_2 production was determined under the influence of lights of different wave-lengths, the procedure was essentially the same. The animal was allowed to remain quietly in darkness for fifteen minutes. The motor was started and determinations made in the darkness until two which checked closely were obtained. The stopcocks were then adjusted, the stop watch started, and the frog exposed to the light. Usually two observations were made in the light after which it was extinguished and two more readings made of the rate of CO_2 production of the frog in darkness.

The results of these experiments are summarized in the following table. The plus sign here means that the time required for the change in pH was longer in the light than in darkness, the minus sign that it was shorter.

It may be seen that there is very little variation in the rate of CO_2 production in darkness and in light, and that the differences, with perhaps three exceptions, are within the normal variation. Furthermore, the

variation is not consistent in its direction. The rate of production in light is sometimes greater and sometimes less than that in darkness. It is concluded in agreement with Ewald that light, per se, has no effect upon the metabolism of the frog, as shown by the excretion of CO_2. But as Krogh (7) has pointed out, experiments such as these can not be considered as

TABLE 5

Time necessary for change of pH 7.3–7.0

FROG X (TEMPERATURE 23°–23°C. RESTLESS)	FROG XV (TEMPERATURE 22.5°–22.5°C. QUIET)
2′9″	2′40″
3′7″	2′38″
2′27″	2′41″
2′43″	2′40″
2′13″	
3′5″	
2′39″	
Greatest variation 58″	Greatest variation 3″

TABLE 6

MIDDLE POINT OF BAND OF LIGHT	FROG	TEMPERATURE	AVERAGE TIME FOR CHANGE pH 7.3 – 7.0		DIFFERENCE BETWEEN AVERAGE TIME IN LIGHT AND IN DARKNESS
			Darkness	Light	
mμ		°C.			*sec.*
470	VI	21.0	1′27.6″	1′31.5″	3.9
	X	21.0	1′36.7″	1′38.5″	1.8
	X	22.0	59.3″	56.0″	−3.3
	XV	22.0	1′17.0″	1′22.0″	5.0
520	XII	23.0	1′5.0″	1′ 4.0″	−1.0
	XV	22.0	1′25.3″	1′27.0″	1.7
580	X	22.5	1′19.0″	1′18.0″	−1.0
	XII	23.0	1′25.3″	1′21.5″	−3.8
	XV	22.0	1′22.5″	1′24.0″	1.5
		22.0	1′24.0″	1′22.5″	−1.5
620	X	21.0	1′56.0″	1′53.0″	−3.0
	XII	21.0	1′46.5″	1′48.0″	1.5
	XV	22.0	1′44.4″	1′44.0″	−0.4

giving a conclusive answer to the question of the effect of light upon cellular metabolic processes. In order to determine whether radiant energy is able to affect the reactions taking place within the cell, it would be necessary to use small transparent organisms which the radiations could penetrate directly.

SUMMARY AND CONCLUSIONS

Changes in illumination, from light to darkness and the reverse, using white as well as colored light, in common with other forms of stimulation, produce an increase in the rate of respiratory movements of the frog. This increase is not due, however, to a stimulation of the metabolic processes of the animal resulting in the production of more CO_2, but rather to a spread through the nervous system of the impulse initiated by the stimulus. The amount of the effect bears no relation to the wave-length of light. The increase in respiratory rate is very transitory and within two minutes the rate is characteristic of the average rate in light and in darkness. The differential effects of wave-lengths of light can not be determined by their effects on the rate, or depth, of the respiratory movements, nor by the rate of CO_2 production of the frog.

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THE EFFECTS OF MECHANICAL AND CHEMICAL STIMULATION OF THE TRACHEO-BRONCHIAL MUCOUS MEMBRANE

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A number of studies have appeared on the effect of irritant gases on the epithelium of the air passages. The latest of these is by Craigie (1), who has sufficiently reviewed the earlier contributions. They are, however, at such variance on many points, particularly with reference to the rôle of the branches of the vagus nerves in the pulmonary reflex arc, that it appears desirable to reconsider the problem in the light of more detailed anatomical knowledge of the nerve plexuses and terminations within the lungs. Concerning the effects of various types of stimuli in the nasal and pharyngeal passages there is pretty general agreement. We shall therefore confine our attention entirely to the reflexes caused by stimuli applied to the mucosa of the tracheo-bronchial tree. In addition to the effects of chemical stimulation, our observations on stimulation by mechanical means will also be included.

Since our anatomical knowledge of the innervation of the lung is most complete for the rabbit, this animal was used for most of the work. Forty animals were utilized. They were supplemented with a small number of dogs for comparison. It should be stated that, as Mayer, Magne and Plantefol (2) have pointed out, the rabbit is the most sensitive animal for experiments on the pulmonary reflexes, while the dog is the least sensitive. The combination of anatomical knowledge of the nerve supply of the rabbit's lung which appears to be as complete as present neurological technique will permit, together with the sensitiveness to stimulation to which reference has just been made, should give data of value on the problem under consideration, namely, the response of the tracheo-bronchial mucosa to mechanical and chemical stimulation.

Two series of animals were employed. The first series was given urethane by stomach tube, and then given ether until after a tracheal cannula had been introduced and the vagi isolated. The cannula had a side arm for connection with a recording tambour.

In the case of experiments with mechanical stimulation the animal was placed under the fluoroscope and the stimulus was applied directly to the mucosa by means of a fine camel's hair brush attached with wax to the end of a small brass wire. The outlines of the bronchi were usually faintly discernible and the position of the brush with reference to the points of branching of the bronchial tree could be controlled with a fair degree of accuracy. As one of us (3) has shown, it is at these points of bifurcation particularly that the nerve terminations are located.

Chemical stimulation was effected by causing the animal to inhale fumes of ammonia, ether, acetic acid, tobacco smoke and formaldehyde through a cannula. The most effective way of applying the stimulus without causing the apparatus to interfere in the slightest degree with the normal respiration was by drawing the vapor from a bottle into a rubber bulb with a small neck and then gradually expelling it at the free end of the cannula. The animal thus inhaled air strongly mixed with the irritating vapor. This gave a nearly uniform period of stimulation of four to five seconds' duration. The connection with the tambour recorded the result. Precautions were taken to prevent stimulation of the nasal mucosa by the fumes as they were released.

The second series of animals was not given urethane, but only ether until the cannula was inserted and the vagi isolated. They were then kept under light ether anesthesia. These animals gave better results, both with mechanical and chemical stimulation.

Results of mechanical stimulation. We found early in the course of our experiments that it was necessary to differentiate between stimulation of the carina tracheae and the intrapulmonary portions of the bronchi. This was true for the various types of stimuli which we employed. Stimulation of the carina with a brush elicited a vigorous response (figs. 1, 2 and 6), which consisted of a forced expiration or bechic blast. This was usually repeated several times in quick succession. When the carina was locally anesthetized with cocaine or when the vagi were sectioned, no further response from stimulation at this point was obtainable.

Stimulation of the deeper portions of the tracheobronchial tree produced results which were less marked in degree but were similar in type. Proper mechanical stimulation of the intrapulmonary passages in the rabbit is difficult because of the small diameter of these tubes, and the ease with which the mucosa is injured. Hemorrhage is very easily produced and, even when not soon fatal, vitiates the results, apparently by coating the mucosa in such a manner that the latter is not receptive to stimulation. We found that animals with respiratory infection were also refractory, apparently because the mucosa was coated with secretion. It was necessary to have healthy and active animals. In such rabbits, however, mechanical stimulation of the intrapulmonary bronchi produced reflexes

similar to those from the carina but much less pronounced. These could be best studied by observing the movements of the diaphragm under the fluoroscope. These movements consisted of spasmodic expiratory efforts when a sensitive point of the mucosa was stimulated. Stimulation of the division points of the main bronchus near the hilum gave responses much more marked than those obtained deeper within the lung. In a few animals it was possible to insert the brush along the main stem bronchus almost to the diaphragmatic surface of the lung, but no response

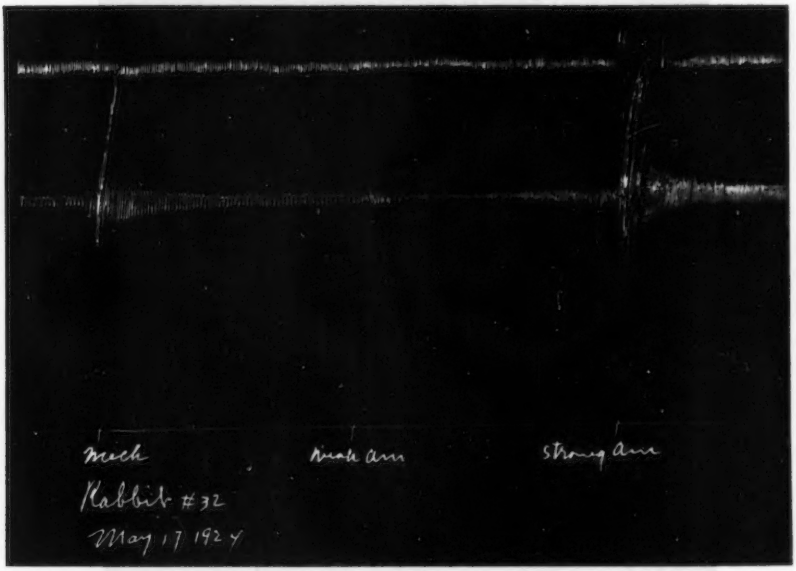

Fig. 1. Result of excitation of lungs of rabbit including carina, with mechanical stimulation, with weak ammonia fumes, and with strong ammonia. Vagi intact. Stimulus applied through tracheal cannula. The animal was under light ether anesthesia.

was obtained below the upper half of this tube. Attempts were made to insert the brush into the primary branches of the main stem bronchus but with only indifferent success in the rabbit. In a few cases there was an apparent slight response, but this was probably due to stimulation of the nerve terminations at the point of division from the main bronchus.

It is an interesting fact that after any of these points were stimulated sufficiently to produce a bechic blast, it was not possible to produce a second reflex from this point again for several minutes (fig. 2). Stimulation of other sensitive areas within the same lung would, however, call

forth the reflex immediately. Chevalier Jackson (4) has reported from his clinical observations phenomena resulting from the entrance into the lungs of foreign bodies of other than organic substance, and also in connection with the insertion of the bronchoscope into the trachea and bronchi. He states that "a fixed foreign body causes very little cough, as compared to a movable foreign body." He also calls attention to the "symptomless interval" after the initial choking and coughing when a

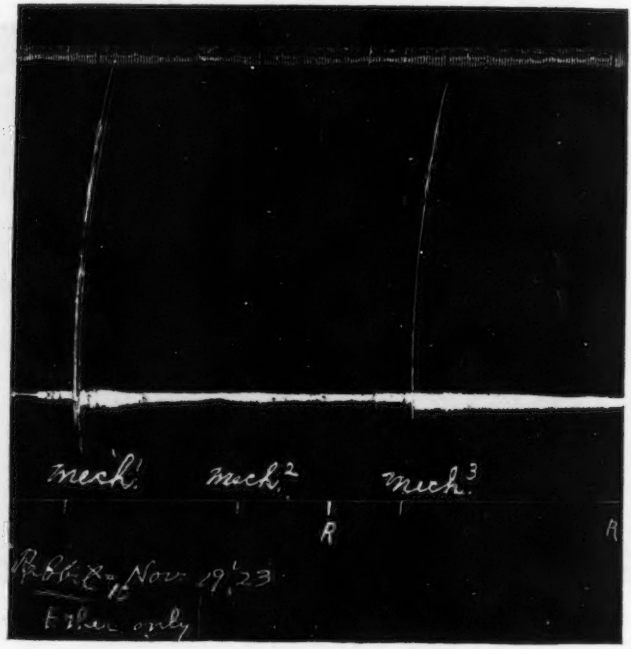

Fig. 2. Result of mechanical stimulation too soon repeated, giving no response the second time, *mech 2*, but after a rest period of about five minutes, *R*, a third application of the stimulus, *mech 3*, gave a marked reflex.

metallic foreign body is aspirated. Jackson attributes the cessation of the cough reflex in such cases to establishment of a "tolerance" by contact with the same mucosal surface over a period of time. Our results however indicate that the reflex is not again elicited for several minutes, usually two to five, in the rabbit even when the brush is withdrawn entirely from the trachea. We are inclined to regard the lack of immediate response as resulting from a fatigue, probably of the receptor mechanism.

Section of the vagus nerves resulted always in lack of response to mechanical stimulation (fig. 3). It is a point of interest that stimulation of the carina after section of one vagus produced varying results. Sometimes a slight response was elicited, sometimes no response, and occasionally a response of considerable violence. This appears to indicate that the right and left vagi share the innervation of the tracheal mucosa in

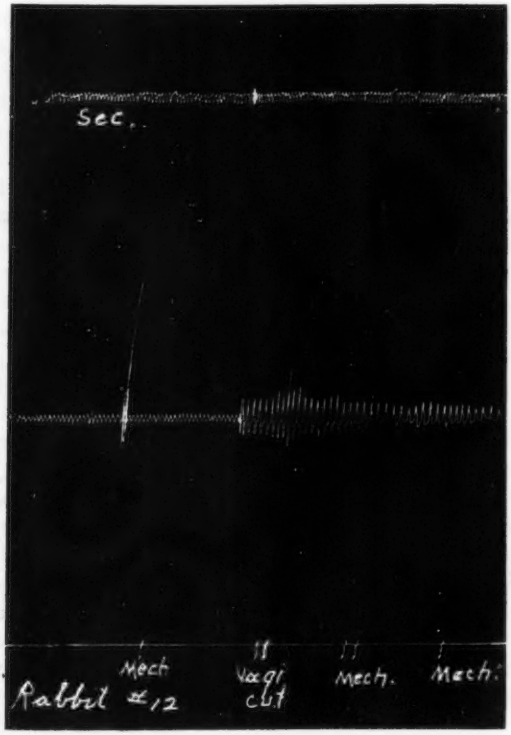

Fig. 3. Result of double vagotomy on response to mechanical stimulus, applied at *mech*. No response.

varying degree. No such results were obtained from the intrapulmonary bronchi, although Schiff (5) long ago pointed out that there is a certain amount of crossing over of the vagi. Larsell and Mason (6) have also observed this anatomically. Evidently, however, it is relatively so limited that stimulation of such crossed-over endings is very rare.

In order to further study the effect of mechanical stimuli within the lungs we employed a bronchoscope on a dog of medium size. The in-

strument was inserted into the trachea about 4 cm. below the larynx and adjusted to various positions within the lungs during the course of the experiment. The animal had been injected intravenously with urethane and was kept under light ether anesthesia in addition. The vagi were isolated preparatory to section. For invaluable assistance in this experiment with the bronchoscope, we are indebted to Dr. Ralph A. Fenton to whom we desire to express our thanks.

Stimulation, with a brush, of the carina produced marked bechic blasts. This was also true when the division points of the main bronchi into the primary branches were touched. A lesser response was induced when the division points of the primary branches into secondary bronchi were stimulated. We were not able to penetrate deeper than these points with the apparatus at hand. No response to stimulation at any of these points was obtained after the vagi were sectioned. This fact, true of both dog and rabbit, is in agreement with the anatomical result reported by Larsell and Mason (6), that nearly all the nerve terminations within the lung disappear after homolateral degeneration of the vagus.

Our experimental results are in harmony in most respects with the clinical observations reported by Chevalier Jackson (4). Both anatomical and experimental results on the rabbit and on the dog so far as they have been checked on the latter animal, show that without question there is present a receptor mechanism in the larger air passages at least, which on mechanical stimulation elicits a forced expiratory response. These receptors are connected with the central nervous system by the vagus nerves. Jackson quotes, with approval, a personal communication from Dr. F. J. Kalteyer that "there is abundant clinical evidence in support of the view that the pulmonary parenchyma is devoid of terminal afferent nerves esential to this reflex arc."

If by "pulmonary parenchyma" is meant only the atria and air-sacs, we would agree with this statement with the reservation that in the atrial walls nerve endings are present whose possible function we will consider below. Larsell (3) has shown that in the rabbit at least there are nerve endings of a type presumably receptive to mechanical stimulation in the air passages as far distally as the beginnings of the alveolar ducts. The general similarity of innervation of the canine and human lung to that of the rabbit in other respects (7), would lead one to suspect that nerve endings are present in the smaller air passages in the dog and human also although they have not yet been demonstrated by histological methods in these forms. It is possible that these terminations are receptive only to other types of stimuli than the tactile involved in the reflexes above described. It is furthermore not impossible that because of their small size and relative isolation one from the other, a large number must be stimulated almost simultaneously, as by collapse of the lung in expiration,

to induce a response. There would thus be produced a sort of summation of stimuli.

Response to chemical stimulation. In studying the effect of this type of irritants on the pulmonary mucosa, we found that fumes of ammonia and acetic acid produced the most marked effects of the irritants employed although ether served very well on animals which had recovered from the anesthetic or were only slightly under its influence.

Brodie and Russell (8) obtained a cardiac response to chloroform vapor, when applied to the nasal passages and the larynx, and also when applied to the lower respiratory passages. They confirm Kratschmer (9) and Francois-Franck (10) on the nasal passages and the results of the latter in obtaining a reflex cardiac and respiratory inhibition by stimulating the laryngeal mucosa with chloroform vapor. The conclusion of Francois-Franck that the respiratory tract below the vocal cords is insensitive to chemical stimuli, Brodie and Russell are unable to share. They state "We have frequently recorded slowing as an immediate result of chloroform administration. There is no question that this part of the respiratory tract is less sensitive than the larynx and nasal mucous membranes. In fact as we proceed downwards along the respiratory tract the mucous membrane is found to be less and less sensitive to chemical irritants."

When our rabbits inhaled ammonia, ether or acetic acid fumes through a tracheal cannula, there ensued a violent expiratory reflex (figs. 1 and 4) which was repeated a number of times if the animal was particularly sensitive. This was frequently followed by a period of apnea of from two to five seconds when ammonia fumes were introduced. This in turn was followed by marked polypnea often continued for many minutes, but we never observed a case continuing for as long as half an hour, as reported by Mayer, Magne and Plantefol (2). When fumes of weak ammonia were employed all response was absent (fig. 1).

Inspiration of acetic acid vapor incited a similar violent cough (fig.4) which was always followed by the polypnea without an intervening period of apnea. Ether gave results (fig. 5) similar to those of acetic acid. With irritant vapors, as with the mechanical stimulus, a rest period of two to five minutes was necessary between separate applications or failure of the reflex resulted. Double vagotomy is followed by lack of response to stimulation in all cases (figs. 4 and 5).

In an effort to analyze the effect on the deeper bronchi alone we carefully cocainized the trachea and the carina. After these parts had become so anesthetic that they failed to respond to stimulation with the brush we felt reasonably certain that any effect of chemical irritation must be due to stimulation of the deeper air passages.

The reflex response after this procedure was not so strong, but was almost always easily induced (fig. 6). It consisted in these cases of one

or more bechic blasts, which were followed, irrespective of the vapor employed, by a polypnea. No period of apnea such as that following administration of ammonia fumes when the carina was not cocainized, was ever observed. The response invariably disappeared after double vagotomy.

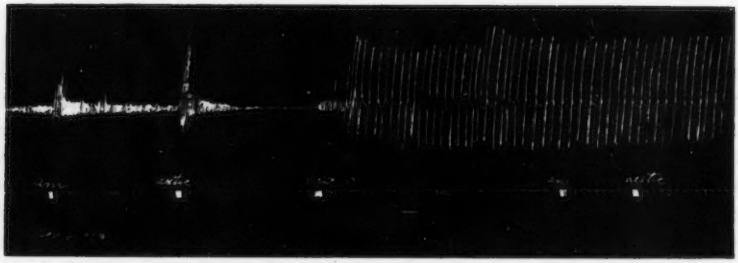

Fig. 4. Result of stimulation with vapor of ammonia and of acetic acid before and after double vagotomy. Animal under light ether anesthesia.

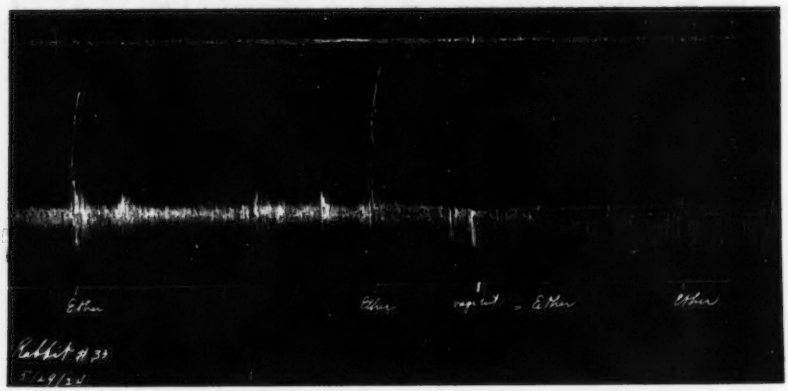

Fig. 5. Result of stimulation with ether before and after double vagotomy. Animal had been allowed to partially emerge from the deep ether anesthesia which was used while the operative work was done.

Brodie and Russell (8) state that chemical "stimulation of the alveolar nerves is about as effective as that of the laryngeal." On histological grounds the only nerve endings which can be regarded as "alveolar" in any strict sense are those located in the walls of the atrial spaces (3). No terminations have been found in the air-sacs proper. It is possible that these atrial terminations are receptors of chemical sense, as indeed one might suspect from their structure and position. One is tempted to

suggest that it is these terminations which account for the results of Suñer (11), (12) and Suñer and Bellido (13). These workers supplied the respiratory center of an experimental dog from the blood stream of an animal not further treated. When the experimental animal was caused to inhale various concentrations of CO_2 gas, respiratory effects similar to those usually attributed to the respiratory center were observed. These effects were no longer produced after section of the vagus nerves.

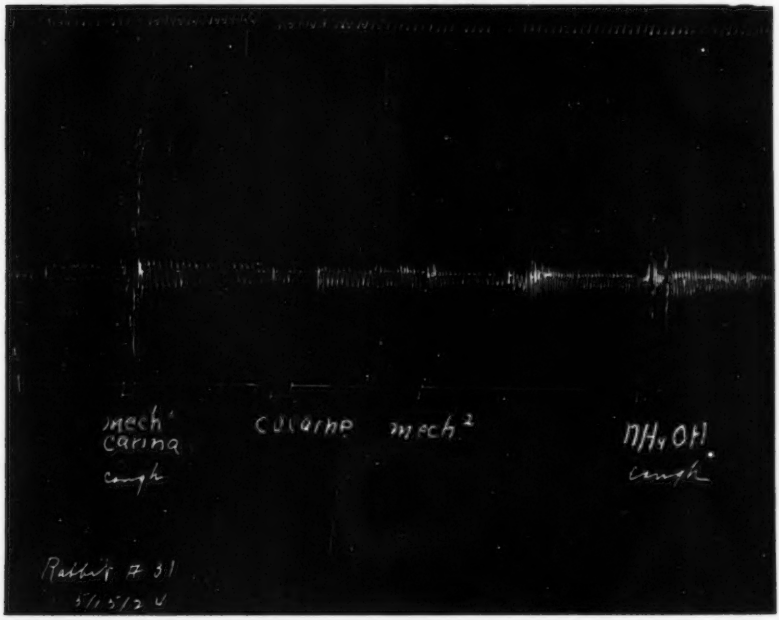

Fig. 6. Result of stimulation of deep air passages with ammonia fumes after the trachea and carina had been treated with cocaine. The preliminary mechanical stimulus, *mech 1*, was to test the sensitiveness of the animal. The second, *mech 2*, was to test the effect of the cocaine on the trachea and carina.

Whether or not it is these endings which were stimulated by the ammonia and the other vapors employed in the present experiments, we have no means of knowing. Anatomical conditions and experimental results are however in close harmony with the conclusions of Suñer (11) that the lung or the final bronchial ramifications are sensitive to certain chemical excitations which cause respiratory reflexes carried by the vagus.

In the main our results with rabbits are similar to those reported by Mayer, Magne and Plantefol (2). We would call attention especially to

the sharp expiratory blast, which these authors as well as we also observed, and to the cessation of the response after double vagotomy, in which again we find ourselves in agreement with the authors named. On both these points we are in disagreement with Roger (14), who also worked on rabbits. Roger's tracings show arrest of respiration in the inspiratory phase after projection of irritant vapors into the trachea, and he states that double vagotomy does not affect the response. Craigie (1), working with dogs, states concerning the reflexes produced by blowing gas into the trachea and bronchi that "These were found to conform more nearly to the description of Mayer, Magne and Plantefol than to that of Roger." This statement is borne out by his tracings which, for normal animals, are very similar to ours, even to the brief period of apnea when ammonia was used. Craigie also states that the reaction commences with an expiratory spasm, and refers to the period of apnea.

He states however that "Both the respiratory and circulatory reflexes are absolutely unaffected by double vagotomy," thus agreeing with Roger so far as the respiratory effect is concerned. Craigie's tracings are far from convincing on this point. His figure 2 B certainly does not show the reflex effect and polypnea so manifest in figure 2 A, although the so-called vagus breathing is very evident. His figure 3 also makes his statement appear over-emphatic. After making due allowance for the difference of animal, and the possibly greater sensory innervation of the lung of the dog from the sympathetic trunk, which we had been led to suspect on anatomical grounds, we should interpret Craigie's results as indicating agreement with our own in the rabbit, that section of the vagus does materially affect the respiratory reflexes, even in the dog. This conclusion is strengthened by the few experiments we ourselves have made on dogs with irritating vapors. Regarding Roger's statement on this point, as it affects rabbits, we have no suggestion to offer.

CONCLUSIONS

1. Mechanical stimulation of the lower respiratory passages including the carina and the bronchi as far as those of the second order, at least, elicits a marked bechic blast or cough.

2. When the trachea or carina are anesthetized with cocaine the intrapulmonary air passages still respond to mechanical stimulation, but the reflex is less violent.

3. Stimulation of the air passages with irritating vapors, as fumes of ammonia, ether, acetic acid, etc., produces violent bechic reflexes.

4. As with the response due to mechanical stimulation these reflexes may still be elicited from the intrapulmonary air passages when the trachea and carina are rendered anesthetic. Under these conditions the response is less violent.

5. Double vagotomy is followed by failure of response to any of the stimuli above named. It appears therefore that sensory terminations within the lung and in the epithelium of the carina are chiefly responsible for initiating the reflexes involved, and that these endings are connected with nerve fibers which pass through the vagus nerves to the medulla oblongata.

6. The deeper air passages give less marked response both to mechanical and chemical stimulation than do the larger bronchi and particularly the carina.

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THE SYNTHESIS OF VITAMIN C BY GERMINATION[1]

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Since the classical work of Holst and Fröhlich in 1907 (1), the production of vitamin C by seeds during the process of germination has received considerable attention. Fürst (2) in 1912 germinated green and yellow peas, barley, oats and lentils at room temperature for 48 to 72 hours with frequent stirring after a preliminary soaking in water for 24 hours. The water in which the seeds were soaked was poured away and the germinated seeds were fed, to the exclusion of other food additions with the exception of distilled water. He was particularly interested in this problem on account of the work of Holst and Fröhlich (1) who had expressed the possibility that the antiscorbutic activity of certain foodstuffs was attributable to the presence of enzymes. Besides determining the antiscorbutic value of the seeds, Fürst (2) accordingly sought to establish if processes which destroy enzymes also completely destroy the antiscorbutic property, and, furthermore, if the antiscorbutic property would persist after certain operations which did not destroy enzymes. He did not go into details in regard to what special enzymic properties he had in mind,—all of his work is essentially of a prospecting nature.

His results show that all of the aforementioned seeds developed antiscorbutic properties with germination, and in the case of barley he found that this process of germination must be extended beyond the mere preliminary soaking process of 24 hours because barley thus treated was found to have no antiscorbutic properties. He also found that cooking which is known to destroy enzymes did not destroy completely all of the antiscorbutic vitamin and that germinated seeds dried and ground, and then soaked in water, did not give evidence of the presence of vitamin C although known to contain enzymes.

Chick and Hume in 1917 (3) became interested in the production of vitamin C by the process of germination from an intensely practical standpoint. In quest of a suitable source of vitamin C for army rations they repeated the work of Fürst with slight modifications. In the first place,

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they work only with lentils and peas soaked for 24 hours and followed by a germination period of 24 to 48 hours. During this time the seeds were kept loosely packed in funnels to provide access of air in place of the frequent stirring as carried out by Fürst. In the second place, when feeding the seeds, they provided in addition milk previously heated at 120°C. for one hour to inprove the general condition of the animals. They came to the conclusion that germinated seeds were a valuable supplement to the army ration as the source of an antiscorbutic.

Acting on the findings of Chick and Hume (3), Wiltshire (4) demonstrated in service with Serbian soldiers that germinated haricot beans were more efficient in 4 ounce doses (dry weight) in curing scurvy in man than an equal amount of lemon juice. The seeds that he used were also soaked 24 hours and then germinated 48 hours. Comvie (5) also, in 1920, found germinated peas and germinated beans good antiscorbutics.

In 1919 detailed results in addition to those published by Chick and Hume were published by Chick and Delf (6). They introduced the further improvement over the technique of Holst and colleagues of keeping record of uneaten food residues but they also indulge in the rather questionable procedure of Chick and Hume of furnishing their animals in many cases with milk autoclaved for 1 hour at 120°C. They report that such feeding of milk does not affect appreciably the dietary supply of antiscorbutic accessory factor and that it unquestionably facilitates growth when the antiscorbutic vitamin is furnished. The germinated or soaked seeds were fed on top of a ration of oats, bran and in most cases milk. Results are reported giving detailed information in all cases in regard to daily average consumption of milk, oats and bran and seeds.

They found that the soaking of peas and lentils for 24 hours had little, if any, effect upon their content of antiscorbutic vitamin. The process of germination, however, increased the antiscorbutic value of the seed 3- to 6-fold in 48 hours, making them comparable in their content of antiscorbutic vitamin, according to their statements, to that of many fresh vegetables. It is interesting to note that a dose of 7 to 10 grams of peas germinated in the dark was as efficient as an approximately equal dose of peas germinated under ordinary conditions.

Weil (7) and co-workers, working with oats and barley, report that with long continued germination, vitamin C is produced, but they take exception to the statement of Fürst that the antiscorbutic substance is produced in cereal grains during a 3-day period of germination.

The experiments of the authors were initiated in an attempt to determine if the entire process of germination is essential to the production of the antiscorbutic vitamin because it was considered possible, in view of the lability of the vitamin to oxygen, that it had its origin in strictly anaerobic processes.

Of the nature of the changes taking place during germination little more than quantitative data in regard to what is revealed by analysis at different stages of germination is known. It is known for example that in fatty seeds carbohydrates are synthesized, that in general the amino and soluble nitrogen and soluble carbohydrates are increased, that enzymes make their appearance and that there is a great increase in respiratory activity in all germinating processes but the interrelationships of the various processes, the counterparts of those reactions, oxidative and reductive, which are now, among other things, attracting so much attention in animal chemistry, present an unexplored field.

The development by Bezssonov (8) of a reaction of polyphenolic compounds for the detection of vitamin C presented alluring possibilities, especially in view of its lability to oxygen. Unfortunately it apparently has been shown by Kay and Zilva (9) that a positive reaction to this reagent does not parallel the occurrence of vitamin C as closely as Bezssonov's results indicated.

In the animal field one of the interesting problems in relation to vitamin C is presented by the fact discovered by Parsons (10) that in the absence of vitamin C from the diet of the rat, and possibly of other animals as well, vitamin C nevertheless persists in their livers, and no pathological evidence of the need of additional amounts of C is later revealed. It is, however, to be remembered that in all experiments carried out up to the present time, grains were always a component part of the diets of the rats used in these experiments. This suggests the possibility that probably the rat synthesizes vitamin C from certain constituents of the grains just as it has been alleged by the aforementioned investigators that grains produce the vitamin by germinating. In view of this possibility it seems necessary that rats should be fed on rations composed of other constituents than grains for a protracted period of time to see if vitamin C will still persist in their livers under these conditions; and it also suggests that the process of germination be studied in greater detail. Among experiments projected and already initiated along these lines the present report concerns itself with attempts to determine if the prolonged soaking of seeds under anaerobic conditions will allow the synthesis of vitamin C to take place.

Experimental. In our attack of the problem outlined it was necessary, first of all, to verify the fact that germination brings about a synthesis of the antiscorbutic vitamin—not necessarily because of doubt as to the validity of the findings of the investigators already mentioned but because in all work of this nature it is necessary to control the variations in the reactions of animals due to difference in stock and different states of nutrition. As to the latter we had occasion to have its importance reëmphasized to us in some of our experiments when scurvy suddenly became incident in our guinea pigs a few days after they had been started on one of

our rations. Ordinarily in our experience it does not make its appearance much before the 14th day of the experiment. Upon looking up the probable cause of this variation we found that the difficulty lay in depending upon old white carrots (11) as the source of antiscorbutic vitamin for our stock. While our stock outwardly did not show any signs of scurvy, nevertheless it was on the borderline. In the light of results published by Parsons and Reynolds (12) and unpublished results of this laboratory, this situation is understandable because it harmonizes with the depletion of the body tissues in antiscorbutic content.

Our experiments were initiated using both peas and barley. The peas were small yellow peas known as Canadian field peas and the barley was ordinary barley obtained from a commercial seed house. Our experiments with peas were, however, not carried very far because of the difficulty of controlling the growth of moulds. Barley did not appear to be such a good medium for their development.

The barley was fed dry, soaked for 24 hours, then germinated in the dark for 3 days and soaked for 96 hours in the absence of oxygen. When fed dry it was finely ground, when fed soaked for 24 hours it was fed whole, when germinated it was chopped up in a chopping bowl and when soaked for 96 hours it was fed whole to the first 4 animals and later fed chopped in a meat cutter. During health the animals consumed all readily.

In order to provide the animals with a balanced ration there was added for each 100 grams of dry barley a supplement consisting of dried alfalfa 19 grams, casein 6.35 grams, sodium chloride 0.63 gram and calcium carbonate 1.27 grams, which was made up in bulk. This was readily incorporated with the barley,—in case of the soaked or germinated grain after such soaking or germination on the original 100 gram dry basis—and was readily consumed by the animals. The supplement also served the purpose of absorbing the water in which the grain had been soaked. With the germinated seeds, however, this was lost as after the original soaking of 24 hours in 125 cc. of distilled water, the seeds were poured on a 4-inch funnel covered with a doubled layer of cheesecloth to provide moisture and allow access of oxygen. The seeds were moistened daily by pouring water on them and were in addition kept from drying by a funnel inverted over them. As positive results were obtained in spite of the loss of this water it evidently was not a factor of importance as a carrier of the vitamin.

We paid particular attention to getting accurate consumption records in daily or 2-day periods, all records except the dry barley being referred to a dry-weight basis. The figures for dry barley are given on an air-dried basis. The former were obtained by removing the feed residues from the feeding crocks and drying them at 86°C. in individual paper sacks for 4 to 7 days. For comparison a number of feed portions mixed with supplement were dried for an equal period. The necessity of securing consumption records is further imposed by the fact that a loss in dry matter

results by the metabolic activity during germination, which presents the possibility of concentration of vitamin as well as increased consumption due to greater palatability. We proved to our satisfaction that this loss in dry matter is not of much moment from the standpoint of the results obtained—being of a magnitude less than 10 per cent of the total fed. Both soaked and germinated barley were readily consumed.

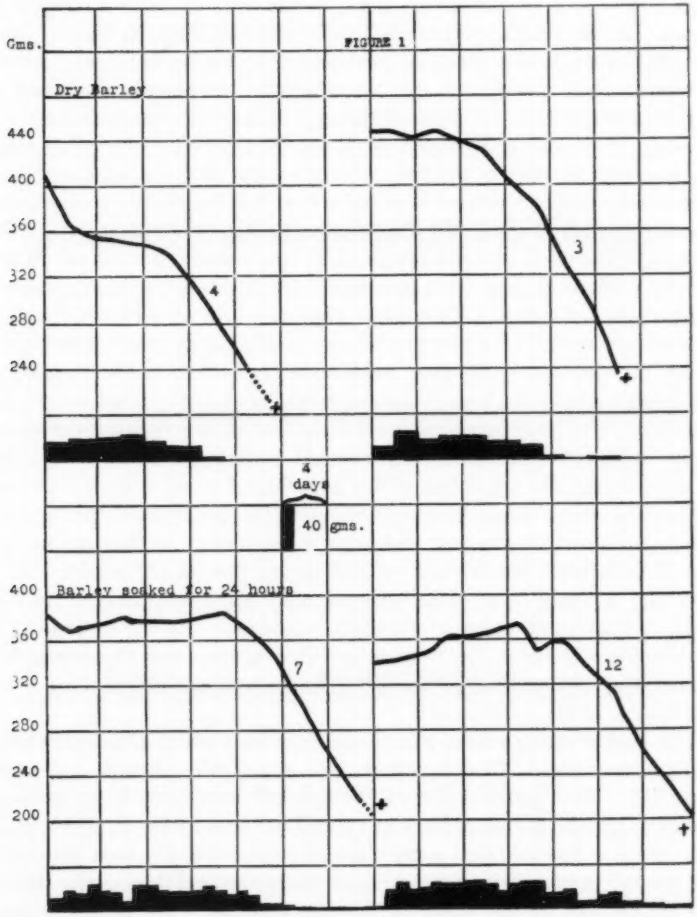
The securing of accurate consumption records was made convenient by taking advantage of the technique introduced by Steenbock, Sell and Nelson (13) of keeping the animals segregated in cages provided with false screen bottoms. This eliminates the necessity of using absorbent bedding and allows immediate detection of the spilling of any of the ration. The ration was fed in earthenware crocks 4 inches in diameter and 3 inches deep which were anchored to the screen floor by wire stirrups slipped over the top. This stirrup furthermore prevented the animals from getting into the crocks or pawing out the ration. Water was provided in similar containers.

The guinea pigs were weighed at least once every 2 or 3 days and careful note made of their activity, swelling of the wrists, symptoms of pain, diarrhea, etc. After death all animals were examined for hemorrhages, intramuscular, subcutaneous and intestinal, and also for loosening of the teeth and enlargement of costo-chondral junctions and wrists.

Results of feeding the dry barley ration are shown in the upper part of figure 1. Guinea pig 3 had swollen wrists after having been on the ration for 12 days. Upon death at the end of 22 days post mortem showed a severely hemorrhagic intestine and both costo-chondral junctions and wrists were more or less congested. Guinea pig 4 had swollen wrists at the end of 16 days. Its history was slightly complicated by an early abortion which took place 6 days after the beginning of the experiment. Its symptoms of scurvy as revealed by gross examination were not pronounced but post mortem upon death after 19 days showed that its teeth were loose and wrists and costo-chondrol junctions were hemorrhagic. The food consumed by pig 3 in the first 15 days averaged 16 grams. Pig 4 in an equal period of time consumed on an average 15 grams. In both cases the weight of food consumed in this period was very uniform. The initial loss in weight can therefore not have been due to starvation, it without doubt can be directly attributed to the incidence of scurvy.

Two other pigs were run later but these were started from our stock at a time when it was being fed carrots as a source of antiscorbutic vitamin as already stated. These animals gave indications of the prevalence of scurvy after 8 days and died after being fed the ration for 17 and 18 days respectively. Both revealed a decided scurvy condition upon post mortem as indicated by loose teeth and swollen and hemorrhagic wrists. Two days before death after having been on the ration for 6 days their weights

had fallen from 455 to 260 grams and 387 to 240 grams respectively. On the average for this period they had consumed 11.5 and 11.2 grams of ration daily.



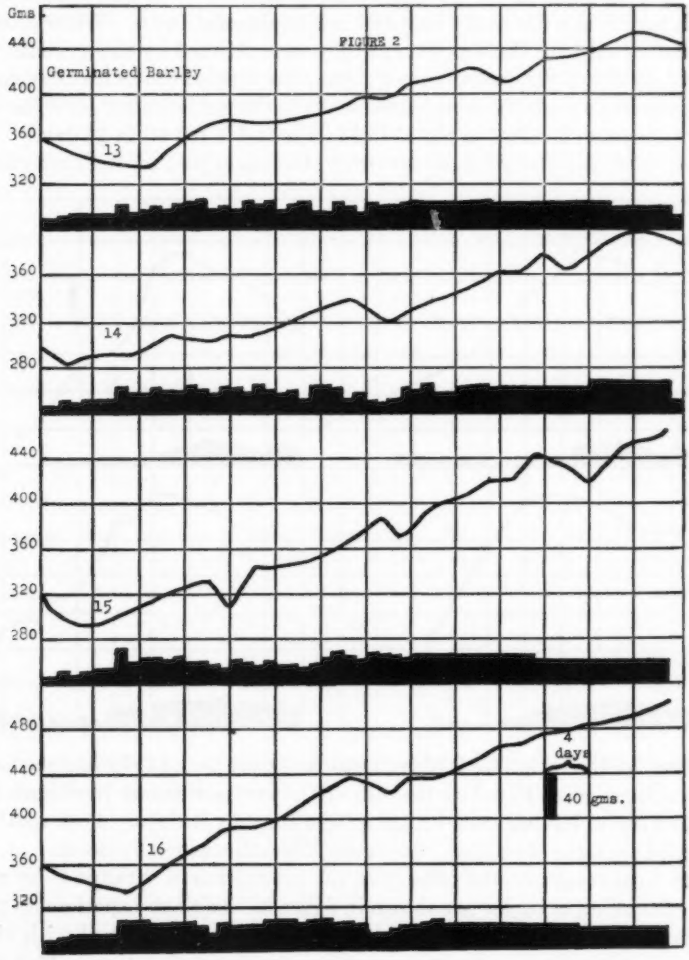
The results obtained with barley soaked for 24 hours are shown in the lower part of figure 1. Guinea pig 7 gave indications of being afflicted with scurvy by its general lethargic condition and by its swollen wrists by the 18th day. After having been on the ration for 28 days it died. Post mortem revealed hemorrhages at the wrists and a slightly congested colon.

Pig 12 showed the first signs of scurvy on the 16th day by its general inactivity. It died on the 30th day. Post mortem revealed loose molars, intramuscular hemorrhages at the knees and slight congestion of the colon. The food consumed by pig 7 in the first 18 days averaged 17.7 grams. In the similar period pig 12 consumed 23.2 grams daily. As the chart indicates, loss of weight again began before the occurrence of a reduction in food consumed. On the whole the performance of these animals was better than of those on the dry barley, but no importance can be assigned to this comparison from the standpoint of the comparative antiscorbutic potency of the two rations because of the incidence of scurvy in each case within the time limits in which it is frequently found to occur when animals are kept on the same ration. It is certain that the synthesis of the antiscorbutic vitamin resulting by the soaking of seeds for 24 hours is of such degree, if any, that it does not allow of unequivocal demonstration by our technique. Two other pigs were run on this same ration at another time, but like the two mentioned in connection with the dry barley rations, scurvy became incident in one within the first week and in the other a few days later, but unlike pigs 7 and 12 they lost weight much more rapidly. The curves for these are not shown because the animals apparently were not sufficiently stocked with reserves of antiscorbutic vitamin before the beginning of the experiment to be considered normal.

Figure 2 shows the results obtained with the germinated barley. Four pigs maintained themselves and grew consistently on this ration for 55 and 57 days when the feeding of this ration was discontinued. At this time the animals were of an exceptionally sleek appearance and gave every indication of being in excellent condition. As the records of food consumption indicate, pig 13 averaged 20.5 grams; pig 14 averaged 21.4 grams; pig 15, 21.7 grams; and pig 17, 22 grams of ration daily for the entire time of the experiment.

The barley sprouts were practically free from green pigment when fed and photosynthesis of compounds was practically though not entirely excluded. This qualification of the general statement is made because even though the germination was carried out in a locker in absolute darkness, yet it is possible that some photosynthetic activity took place during the time of preparation and mixing of the ration and during the time that the ration was in the cages before the animals. We doubt, however, if this can have been serious matter because the animals were fed in a dimly lighted room and the seeds were fairly well macerated. In the synthesis of vitamin C in darkness our experience confirms the experience of Chick and Delf (6) who worked with peas. By comparison of the results obtained with the animals on dry or soaked barley, there remains no question but that synthesis of the antiscorbutic vitamin by the sum total of processes taking place in germination occurs.

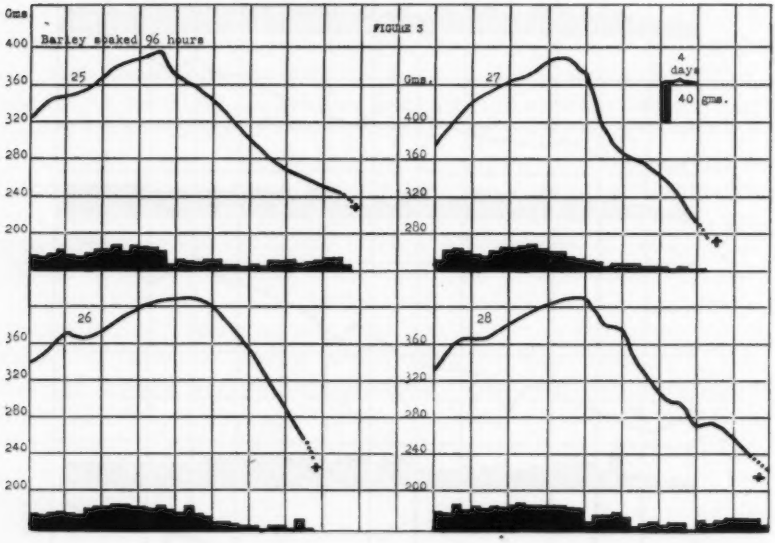
With the termination of the above experiment the animals were used to obtain preliminary data on the effect of prolonged soaking of seeds in the absence of oxygen on the production of vitamin C. For these experi-



ments the seeds were soaked in 100 gram quantities with 125 cc. of distilled water in ½-pint milk bottles. To allow escape of gas with change of temperature and yet not allow the entrance of oxygen from the air the bottles were stoppered with cork stoppers provided with Bunsen valves. They were kept in a dark locker. At the end of 96 hours the seeds were

found to have imbibed a large amount of water and evidently considerable hydrolysis of their constituents had taken place, but they did not show the slightest signs of germinating,—this process having been inhibited by the early exhaustion of the supply of oxygen. The seeds had a slight sour mash odor and a distinctly sour but not unpleasant taste. When ground up and mixed with the supplement they were relished by the animals.

The results of our preliminary experiments showed that with the change of the animals from the germinated seeds to the seeds soaked for 96 hours, scurvy became incident in pig 15 in 14 days and in pig 16 in 16 days. Pig 13 revealed no outward signs of scurvy, but upon post mortem after hav-



ing been on the ration for 20 days its molars were found to be loosened and hemorrhages were found at the site of the costo-chondral junctions. Pig 14 died early, having been on the ration for only 9 days. Post mortem revealed no signs of scurvy. Its cause of death was not evident.

The final results on the efficacy of the production of vitamin C by prolonged soaking of barley are shown in figure 3. In these experiments particular attention was paid to having the animals well stocked with vitamin C before they were put upon the experimental ration. This was done by feeding the pigs all the fresh green grass which they would consume in addition to a grain ration of rolled oats and water. They were kept on this preliminary ration for a week to 10 days. When changed to the barley ration there resulted no failing of appetite. They started to lose weight at about the 16th day as the graphs in figure 3 indicate and si-

multaneously with this loss in weight scurvy was revealed by the occurrence of swelling of the wrists in 3 of the animals. The animals also became exceedingly inactive. By the 37th day all had died. On post mortem pigs 25 and 26 showed intramuscular hemorrhages and enlarged and congested costo-chondral junctions; pig 28 revealed severe intra-muscular hemorrhages; but pig 27 had only loose teeth. It showed no enlarged joints, no subcutaneous hemorrhages nor intra-muscular hemorrhages,—except one, which may have been a bruise, and its intestines were normal. Though undoubtedly scorbutic, its case was not clear-cut.

By way of verification, 4 other guinea pigs weighing from 315 to 335 grams and previously fed on grass as before were put on barley soaked for 96 hours. With good consumption of ration, 2 developed swollen wrists in 17 days and one in 21 days; the fourth did not develop any gross signs of scurvy but by the 22nd day on post mortem,—at which time all the others had already died—it was found to have subcutaneous hemorrhages. The others had, in addition to swollen and hemorrhagic wrists, subcutaneous and intra-muscular hemorrhages and one had hemorrhagic costo-chondral junctions.

SUMMARY

Vitamin C is synthesized in considerable amounts by the barley kernel during germination. This takes place even in the dark. It does not, however, take place to any appreciable degree during the first 24 hours of soaking, or even in 96 hours when carried out in the absence of oxygen. From this it appears that vitamin C, though easily destroyed by oxygen nevertheless requires oxygen for its synthesis by the germinating seed. Attention is called to the necessity of studying the synthesis of vitamin C from precursors in the seed to determine if animals which do not need vitamin C nevertheless need such precursors for its synthesis. Attention is also called to the danger of using guinea pigs, depleted in vitamin C without actually showing scurvy, as subjects for studies in the occurrence of this vitamin.

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THE RELATIONSHIP OF PHOSPHATE AND CARBOHYDRATE METABOLISM

II. The Effect of Adrenalin and Phloridzin on the Excretion of Phosphate

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In a previous paper (1) were described the changes in excretion of phosphate following the administration of insulin and the ingestion of sugar. These changes were so definite and constant that a further investigation was undertaken to learn if similar changes would be manifest if the metabolism of sugar were altered by other agencies. The well known effects of adrenalin and phloridzin were first studied.

Mention of the effect of adrenalin has been made by Perlzweig, Lathan and Keefer (2) and by Winter and Smith (3), but their results have not been entirely constant. The former state that a subcutaneous injection of adrenalin in normal human subjects caused a fall in inorganic phosphate of the blood, and a simultaneous fall in the rate of excretion of phosphorus in the urine, though frequently there was a rise in the rate of urinary excretion of phosphorus. The latter also found that adrenalin caused a fall in phosphate when the blood sugar was rising above normal; if given during insulin hypoglycemia, the phosphate as a rule rose at first with the blood sugar and later fell.

The methods used in this investigation were the same as in the previous work. Dogs were fasted during the course of the experiments in order to avoid the influence of food. This is very desirable, as under these conditions the excretion is very constant from day to day, and the normal diurnal rhythm is not affected by meals. The animals were catheterized at three-hour intervals during the day, and the twelve-hour night urine was collected as a whole. Fifty cubic centimeters of water were given by stomach tube at the beginning of each day period and 150 cc. at night. Analysis of the urine was made for inorganic phosphate, total nitrogen and sugar, using the methods of Briggs (4), Folin (5) and Shaffer and Hartmann (6) respectively.

Adrenalin and phloridzin were given subcutaneously in single and repeated doses, and on one occasion adrenalin and insulin were given

together. The results are shown in the accompanying graphs. The vertical lines mark off three-hour periods, beginning at 10:00 a.m. and the horizontal lines represent the excretion of each substance per hour, phosphate being expressed in milligrams of phosphorus, and nitrogen and sugar in grams. Phosphorus is shown by ————, nitrogen by ---- and sugar by ooooooo. The total quantities for each day are also recorded in the figures.

RESULTS. *Dog H*, a small female terrier weighing 5.2 kgm. at the beginning of the experiment, was used after a preliminary fast day.

On May 12 and May 13 the fast was continued and the excretion was studied for control. The phosphate showed the normal diurnal rhythm

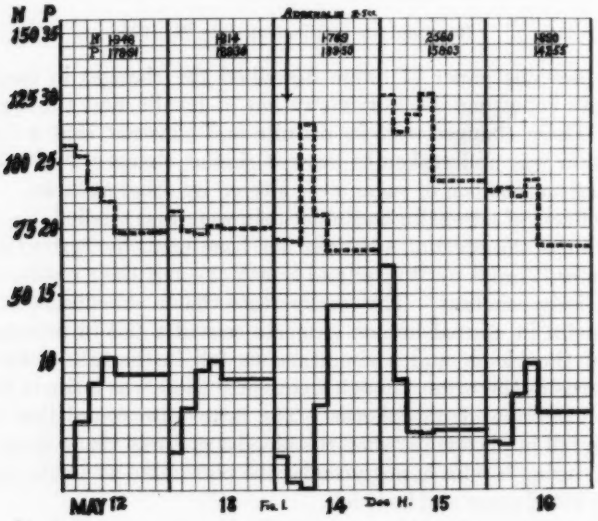

Fig. 1

being very low in the morning and rising toward evening. The nitrogen was fairly constant, though it was considerably higher on the first morning than subsequently. This was probably the effect of the previous feeding.

On May 14, at 12:45 p.m., a single injection of 2.5 cc. of adrenalin (1—1000) was given. There were no immediate effects apparent, but several days later necrosis of the skin, with sloughing, appeared at the site of injection. The phosphate excretion in the first period following the injection, instead of rising as occurred on the normal days, fell from 2.7 mgm. per hour to 0.6 mgm. and in the next period to only a trace. It then rose, reaching a high level during the night, the night urine con-

taining 62 per cent more phosphate than that of the previous two nights. The total for the day was increased 9 per cent. The excretion was still high the following morning but fell to a lower level than normal during the day so that the total for the twenty-four hours was unchanged. The next day the phosphate excretion had resumed its usual diurnal variation and the total output showed the decrease which normally occurs in the course of starvation.

The nitrogen output was markedly increased in the second period after the injection. This increase was only temporary and return to the previous level occurred in the evening so that the total for the day was not changed. The following morning the nitrogen had risen again to a very high level which was maintained during the day. During the night a slight decrease occurred, but the total for the day was 40 per cent above the normal. On the second morning the nitrogen was still somewhat higher than normal, but the total for the day was only slightly increased.

There was not sufficient glycosuria after the injection of the adrenalin to give a positive reaction with Benedict's test, but there was a perceptible increase in the reducing substances of urine determined as sugar by the Shaffer-Hartmann method. The excretion of sugar began in the first period, was at its maximum in the second period and continued in the third period 6 to 9 hours after the injection.

Dog I, a female terrier weighing 7 kilograms, was fasted beginning May 16. On May 17 and 18 the urine was collected for the twenty-four hours, water only being given. On May 19, the urine was collected in the regular three-hour fractions, and adrenalin was given in two doses of 0.5 cc. each. The first injection was made at 1:00 p.m. at the beginning of the second period of the day, and the second dose two hours later. No symptoms were produced except a slight tenderness at the site of the injections.

The phosphate excretion which was 7.4 mgm. per hour in the morning, fell to 3.8 in the first period after adrenalin and to 0.6 mgm. in the next period. Later an enormous increase to 32.3 mgm. per hour occurred and a high excretion continued during the night. The total for the day was increased about 20 per cent. On the two days following the adrenalin injections the phosphate excretion showed the normal diurnal variations, the total for the first day being slightly decreased, compensating partly for the increase on the previous day.

The nitrogen showed a large increase in the second period after the adrenalin, rising from 0.089 gram per hour to 0.143 gram. This increase was only temporary, the output returning to a low level during the night so that the total for the day was not increased. The excretion for the next two days was nearly 10 per cent higher than normal. No glycosuria appeared.

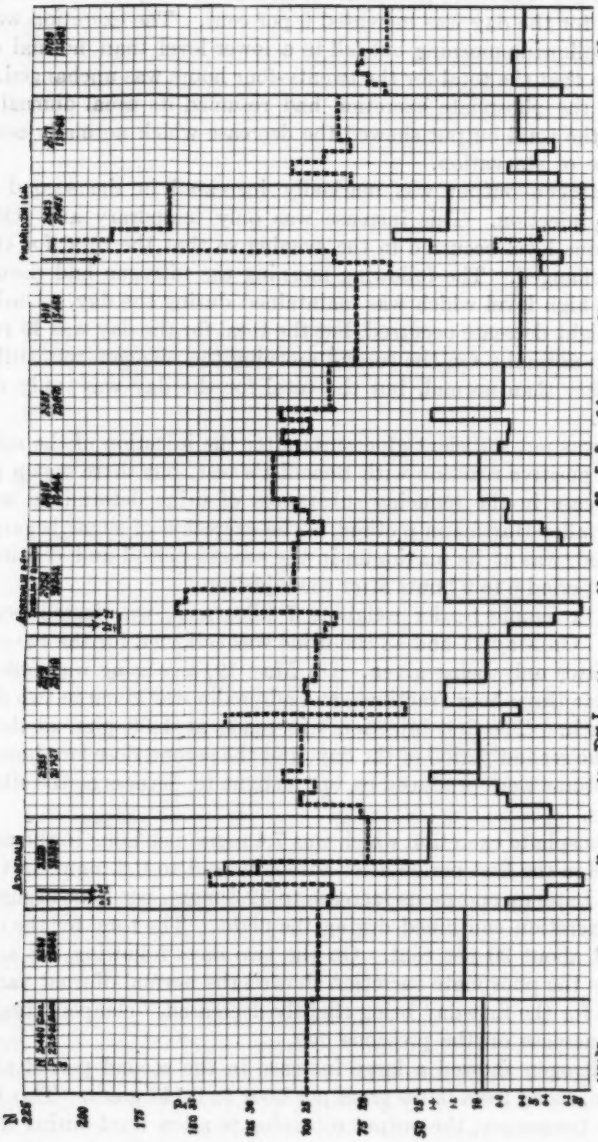

Fig. 2

There was a low output of both phosphorus and nitrogen in the second period on June 21; the reason for this change is not known.

On May 22 the adrenalin was given in exactly the same doses and at the same time but 8 units of insulin were also given at the time of the first injection. The amounts of adrenalin and insulin were calculated to be approximately equivalent. Seven units of insulin given to this dog in a previous experiment had caused similar changes of almost the same magnitude as those caused by the adrenalin on May 19 so that 8 units were given on this occasion.

The phosphate excretion dropped from 7.3 mgm. per hour before the injections to 3.1 in the first period and to 0.9 in the second period following. The subsequent increase reached its maximum during the night the total for the night being increased nearly 100 per cent above the normal night excretion. The increase for the twenty-four hours amounted to about 30 per cent. The following day the excretion had resumed the normal rhythm but the total ouptut was slightly lower than on the next day.

The nitrogen output increased enormously beginning in the second period after the injections when it rose from 0.088 gram per hour to 0.159 and remained about this level for six hours. The night excretion was lower but much above normal as was also the case on the following day, and the first part of the second day. The total for the adrenalin-insulin day was 17 per cent, and for the following day 12 per cent above normal.

On May 26, at 1:55 p.m., 1 gram phloridzin dissolved in 10 cc. of 1 per cent sodium bicarbonate solution was injected subcutaneously. The phosphate showed a slight decrease for the period in which the phloridzin was given, followed by a marked increase which continued during the night so that the total for the day was nearly 80 per cent above normal. The following day the excretion was normal, though the amount was slightly lower than on the next day. The nitrogen showed an extremely large increase, rising from 0.065 gram per hour to 0.90 in the first period after the injection, and reaching 0.215 in the second period. It remained high during the night, but the following day was only slightly above normal. Sugar appeared in the urine of the first period after the injection and reached its maximum in the second period. The night urine contained a small amount, but the morning urine gave a negatve Benedict test. The total sugar excretion amounted to 7.99 gram.

Dog J, a fat spaniel weighing 12 kilograms, was fasted beginning May 20. May 21 and 22 were control days, the urine being collected as usual. On May 23, adrenalin was administered, an injection of 1 cc. being given every hour from 10:00 a.m. to 10:00 p.m. inclusive. No subjective symptoms appeared. Only a trace of sugar shown by Benedict's qualitative test was found in the urine collected at 4:00 p.m. and at 7:00 p.m. and

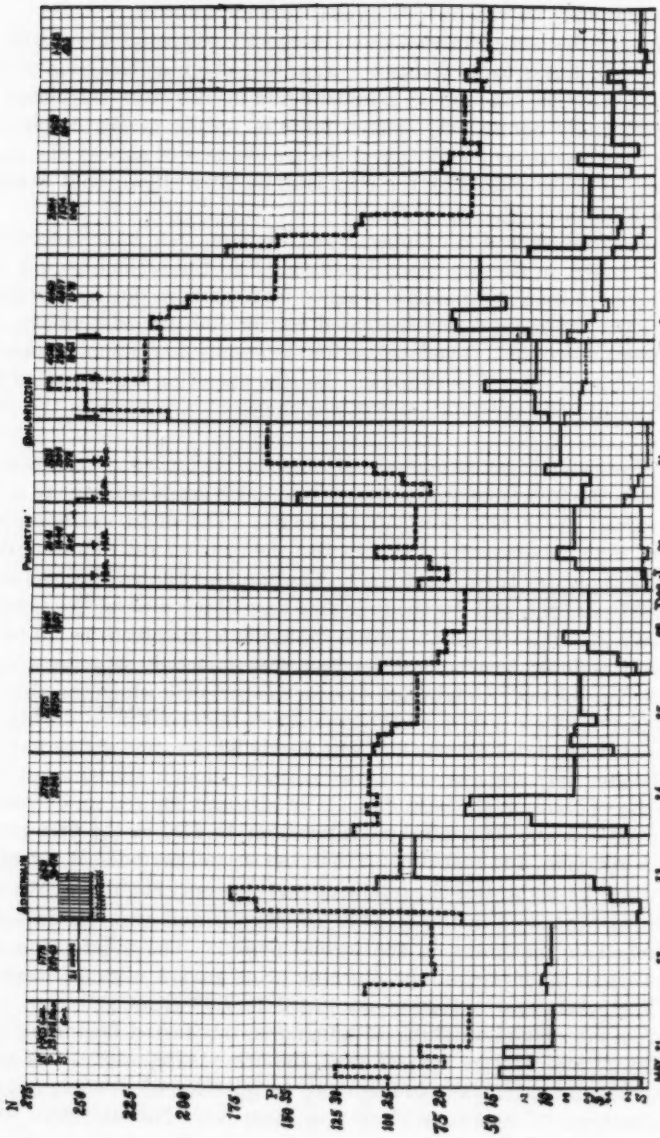

Fig. 3

quantitative estimations were not made. The other specimens gave negative tests.

The phosphate excretion which had averaged 9.4 mgm. per hour in the preceding night was extremely low in the first six hours of the day, 1.2 and 0.9 mgm. per hour. A slight rise to 3.71 and 5.3 occurred in the afternoon and evening but during the night a very high level was reached, the average hourly rate being 22.7 mgm. The output for the day was nearly 50 per cent above normal, that of the following day being also higher. The nitrogen output which was low in the first period showed a large increase in the second and third periods, falling, though not quite to normal, in the evening. The total for the twenty-four hours was increased about 40 per cent and that of the following day about 45 per cent. On the 25th the excretion of both phosphorus and nitrogen had returned to normal.

The fast was continued except that on May 26 the dog was given olive oil. Starting on May 30 and continuing until June 2, 1 gram phloridzin was given twice daily. A phloretin preparation was used for the first three injections, but was not nearly as effective as the phloridzin used for the later injections. The first injection was given at 1:15 p.m., May 30, the second at 10:00 p.m. and one every twelve hours thereafter until 10:00 p.m., June 2.

The phosphate which was quite low on the morning of May 30, remained low for the three hours after the phloretin injection, instead of rising as always occurs normally in the afternoon. Then an increase occurred, not very great however, the total for the day being increased about 11 per cent. On May 31, the phosphate was high in the morning, 6.8 mgm. per hour, and remained about this level until evening when it went up to 10.6. The total for the day was about 50 per cent above normal. On June 1, the excretion was high and fairly constant during the day, the total being increased more than 100 per cent. The effect of the more potent phloridzin is evident here. June 2, the excretion was still higher, the twenty-four-hour output reaching 401.7 mgm. which is approximately three times the normal.

The nitrogen began to increase in the evening of the first phloretin day, the total for the day being increased about 18 per cent. The phloridzin given at 10:00 p.m. May 31 increased the already high nitrogen excretion enormously so that for this day there was an increase of about 80 per cent. The nitrogen continued at a high level for the next two phloridzin days being more than twice the normal.

On June 3 when the phloridzin had been discontinued, the phosphate which was high in the morning, no doubt due to the effect of the injection of the previous evening, fell during the day. On the next two days there was a very great decrease, the output reaching 42.2 mgm. on June 5.

The nitrogen excretion was high in the morning of June 3, but fell during the day until at night it was at a fairly low level. The next two days the output was much lower.

The sugar excretion caused by the phloretin was very small, but the phloridzin brought about intense glycosuria. The D:N ratio on June 1 was 3.64, and on June 2, 3.29.

There were no constant or striking changes in the volume of urine excreted following the adrenalin administration. There was a decrease in the 2nd period after the injections given dog I on May 19 and a slight increase in the same period after the large dose given dog H on May 14. During the third period of the day when dog J received hourly injections the excretion during one period became very high, a compensatory decrease occurring during the night. The changes had apparently no effect on the constituents of the urine. Phloridzin caused a polyuria accompanying the glycosuria, so that there was a slightly negative water balance.

SUMMARY. The results described above may be summarized as follows: The administration of adrenalin to fasting dogs caused a marked decrease in the excretion of inorganic phosphate in the urine, followed by a large increase, so that the total output for the day was increased. The elimination of nitrogen was constantly affected in two phases, first a temporary increase occurring after the injection, when the phosphate was decreased, second a continuous high excretion on the day following. The glycosuria produced was very slight even when the dose was repeated at hourly intervals.

When insulin was given at the same time as adrenalin in an equivalent dose, the same changes occurred, and the joint effect was additive.

An injection of phloridzin, after a slight initial decrease, caused a huge increase in phosphate excretion, lasting about 12 to 15 hours. Repetition of the injections resulted in a continuous high output of phosphorus amounting to two or three times the normal. When the phloridzin was discontinued the excretion became very low probably because of the depletion of the source of phosphates during the diabetic period. The nitrogen excretion increased enormously immediately after the injection and remained high, the effect of a single dose lasting for about 15 hours.

DISCUSSION. It has been shown that the administration of insulin results in a marked decrease in the excretion of phosphates during the hypoglycemic period, followed by an increase, these changes being probably due to the formation of a compound of some product of carbohydrate metabolism and phosphoric acid, which exists temporarily and breaks down, releasing the phosphorus. That adrenalin should affect the phosphate excretion in almost exactly the same manner as insulin, at first sight appears paradoxical, as these two hormones are commonly regarded as having an antagonistic action, on some phases at least, of carbohydrate

metabolism. The occurrence of these changes after ingestion of sugar appears, however, to be analogous, and it is therefore permissible to suppose that some product of carbohydrate metabolism produced by adrenalin has the same effect as the ingestion of glucose.

Insulin and adrenalin cannot be antagonistic in their effect on the process with which phosphates are concerned, whatever that may be, as is shown by the experiment in which insulin and adrenalin were given together. While the amounts of insulin and adrenalin may not have been exactly equivalent, the result must still be regarded as decisive, as the effects were added rather than neutralized.

The increased nitrogen excretion after adrenalin demonstrates that it has an influence on protein metabolism. There has been considerable confusion concerning this question, and Allen (7), after reviewing the literature, states that "since there are so many negative results, it may be concluded that adrenaline has no direct influence upon protein metabolism. The positive reports are perhaps explainable by local tissue necrosis, fever, sweeping out of nitrogen by diuresis, and possibly formation of carbohydrate from protein". The first three explanations cannot be applied in our experiments for although tissue necrosis did occur in one case, it must have been insignificant. Sloughing did not occur until five days after the adrenalin was given, and the increase in nitrogen excretion was not continued beyond the day after the injection. Furthermore, the results in all cases were identical. Our results, supported by the observations of Eppinger, Falta and Rudinger (8), Noel Paton (9) and Underhill and Clossen (10), show that at least in fasting or in under-nourished dogs there is increase in protein breakdown after adrenalin administration. More recently Brel (11) reported that there is a constant rise in the urea of the urine of rabbits, and Marie (12) has found an increased amount of urea in the blood, after injection of adrenalin, which is further evidence in favor of the above hypothesis.

The increase in nitrogen excretion occurred in two phases, first a temporary increase evident in three hours after the injection and lasting three to six hours, and second, a prolonged increase in the level of excretion occurring on the following day. Possibly the first increase is a direct result of adrenalin, causing breakdown of protein to form sugar. The calorigenic action of adrenalin observed by Boothby and Sandiford (13) and by Bru (14), among others, is apparently related to this effect. The prolonged high output may be an indirect effect the result of a demand for carbohydrate to restore the glycogen reserves depleted by the initial effect of the hormone.

The continuous high excretion of phosphate which occurs in phloridzin diabetes, as well as in depancreatized dogs (1) appears to be a significant fact. The phosphorus is not merely a product of the protein catabolism

which is also increased in diabetes, for it is much in excess of that which would accompany the excreted nitrogen in the soft tissues. It must be withdrawn from the reserves in the bone. Perhaps the combustion of carbohydrate exerts in some way a phosphate sparing action analogous to protein sparing action.

An excessive elimination of phosphorus has been observed in human diabetics by several clinical workers, von Moraczewski (15), Mandel and Lusk (16), and von Noorden (17). Falta and Whitney (18) also noted an increase in the excretion of the mineral constituents of the urine of depancreatized dogs, the phosphorus becoming about three times the normal. Yet a definite relationship of the high phosphate excretion to diabetes has not been recognized partly because the accompanying acidosis was held responsible, a view not supported by recent experiments. Forbes and Keith (19), in summing up the evidence, conclude that "The increased phosphorus elimination in diabetes is not a necessary concomitant of the pathological elimination of sugar" and quote the experiment of Lepine and Maltet (20) as proof for this opinion. The latter reported that administration of phloridzin to a dog did not increase the phosphorus excretion but their evidence is valueless for they base their conclusion, not on a study of the actual amount of phosphorus, but of the ratios of phosphoric acid to nitrogen, and to the depression of freezing point of the urine.

Although the question has not been carefully studied, it seems probable that in severe human diabetes there is a high loss of phosphate which may result in an actual deficiency of phosphorus in the body. Even when proper dietetic treatment is carried out, the patient is still likely to have a deficiency, as the limited diet ordinarily required has a low phosphate content, and the depleted reserve of phosphorus in the body will only be slowly restored. The beneficial effect of intravenous administration of phosphate solutions in reducing the hyperglycemia and glycosuria in diabetic patients, which was found by Elias and Weiss, may therefore be due to the supply of an adequate amount of phosphorus.

A report by Smith and West (22) is of interest in this connection. They observed a peculiar symptom complex in diabetic patients—weakness (at times approaching collapse), sweating paraesthesias, stiffness of muscles, attacks resembling mild tetany— not associated with insulin hypoglycemia. These authors attribute the symptoms to a calcium disturbance, as they found a lower calcium content than normal in the blood of these patients. The primary factor may be, however, a disturbance in phosphate, the association of calcium and phosphorus metabolism being well known. Although they did not find the blood phosphorus to be subnormal, a deficiency of phosphorus in the body cannot be left out of consideration. On account of its importance in preserving the pH, the amount in the

blood might still be maintained. It is significant that even when the excretion of phosphorus in the urine has practically ceased as the result of insulin, the percentage in the blood is affected to a comparatively small degree, the average reduction being less than 30 per cent.

The whole question of the requirement of phosphorus in normal nutrition as well as in diabetes requires further investigation. The importance of the mineral elements in metabolism has not been fully recognized and our knowledge of the subject is still very inadequate.

CONCLUSION

The administration of adrenalin to fasting dogs causes changes in the excretion of inorganic phosphate in the urine similar to those caused by administration of insulin or by ingestion of sugar—namely, an initial decrease followed by a large increase. The nitrogen elimination is increased, indicating more active protein metabolism.

Adrenalin and insulin are not antagonistic in their effects on these processes of carbohydrate metabolism, with which phosphate is concerned.

Phloridzin, after a small decrease, causes a marked increase in the excretion of phosphate, the repeated administration of the drug resulting in a continuous excessive loss of phosphate from the body. The question of phosphorus deficiency in diabetes is discussed.

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STUDIES ON VIGOR

I. Effect of Adrenal Extirpation on Activity of the Albino Rat

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Languor and diminution of muscular tone have long been recognized by clinicians (1) as accompaniments of disease of the adrenals, and removal of these glands in most animals is followed by lessened activity and greater susceptibility to fatigue (2). In nearly all species of animals double epinephrectomy proves fatal within a few days (3), (4), (5), (6), the animals manifesting a striking ante-mortem asthenia. As early as 1856–8, however, Harley (7) and Phillipeau (8) independently showed that the white rat may survive this operation in apparently excellent health. It seems to have been generally assumed, and superficial observations bear out the assumption, that in the rats which survive, muscular activity is not significantly altered. The animals appear to be normally alert and the muscular movements seem to be as frequent and extensive as in control animals. The possibility remains open, however, that the normality is only apparent. Careful observation of the habits of caged rats shows that the mere presence of an observer acts for a considerable time as a stimulus. This might serve to evoke an apparently normal degree of activity even in an animal suffering from muscular depression or abnormal fatigability.

Many attempts have been made to create by experimental methods a condition of chronic adrenal deficiency, to simulate Addison's disease or the more or less hypothetical clinical "adrenal adynamia," but without satisfactory results. Cats, dogs and many rabbits, the species that have chiefly been used, seem to be subject to a sort of all-or-none law. If less than about one-sixth of the adrenal tissue is removed hypertrophy of the remaining portion occurs and the animal remains apparently normal. If the adrenal tissue is reduced below a certain critical amount, death promptly ensues. It seemed desirable, therefore, to make a study of the muscular performance of epinephrectomized rats, in the hope that a resulting chronic adynamia could be demonstrated and subjected to quantitative study. Consistent success in this undertaking would seem to open up an important field for investigation.

METHODS. Albino rats of from forty to sixty days were selected in pairs or threes of approximately the same weight and rate of growth and, if possible, from the same litter. Although the rat is almost immune to ordinary infection, it was thought desirable to observe somewhat rigorous aseptic and antiseptic precautions. The hair was clipped from the back over two-thirds of its total area. This area was wet with mercurochrome solution or saturated alcoholic solution of picric acid. Dorsal incisions 1 to 2 cm. in length were made on each side extending downward and outward from the apex of the angle made by the lowest rib and the lumbar muscle mass. After some practice the adrenals could readily be located and drawn up into the incision. In the earlier operations the glands were destroyed with a cautery in order to prevent hemorrhage, but when it was found that hemorrhage is a negligible factor in such an operation the glands were merely snipped out with fine, curve-pointed scissors. The peritoneum-muscle layer and the skin were closed separately with fine silk thread, and small gauze and collodion dressings applied over the skin sutures. Healing *per primam* took place in all instances. Subsequent experience in this laboratory indicates that less elaborate precautions than were employed would have been adequate.

Ether anesthesia was employed at first but in the later operations isoamylethyl barbituric acid (amytal) was given subcutaneously in aqueous solution at the rate of 0.07 gram per kilo of body weight.[1] This resulted in surgical anesthesia in fifteen minutes to half an hour and the animals recovered in about the same time after operation. On account of the relatively narrow margin of safety in this small animal the amount of amytal was subsequently diminished to 0.05 gram per kilo to produce partial narcosis, which was rendered complete by ether as required.

When material allowed, two animals were operated upon for each control, to provide against operative and post-operative fatalities. In the earlier experiments the controls were subjected to identical operative procedures except that a small portion of the kidney was snipped out instead of the adrenal being removed. It was later considered sufficient simulation of the adrenal extirpation simply to make the necessary incisions and pick up the adrenals without injuring them. It should be noted that no difference could be observed in the controls thus differently traumatized. It was found that complete ablation resulted fatally within a few days in about one-third of the animals. Since the controls that had undergone equally severe trauma survived, the fatalities are ascribed to the adrenal deficiency, *per se*.

For measurement of activity two methods were used. In the first, tin-bottomed wire cages six inches square by six inches high were suspended from the lower end of Porter pneumographs hanging on a rigid frame.

[1] We are indebted to Eli Lilly and Company for a generous supply of the drug.

The pneumographs were connected by means of rubber tubing to tambours writing on a drum. This apparatus was sufficiently sensitive to record the slighest observable movements of the animal, even to nibbling a grain of corn.

As Slonaker (9) and others have determined, the white rat at the ages of the subjects of these experiments is predominantly nocturnal in its activities. The activity determinations, therefore, were timed to include the night hours. Records of ten, twelve and fifteen hours were made for fourteen or more successive nights. These included a large proportion of the twenty-four hour activities, since the animals slept most of the day. Through the day the animals were kept in ordinary cages containing food and water. The resulting graphs, with kymograph speed of 3 cm. per hour, give a somewhat variable picture, a typical example of which is reproduced as figure 1. Such records serve to bring out in a convenient

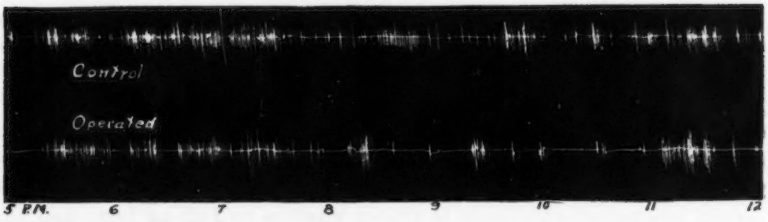

Fig. 1. Kymograph records of activities of control and epinephrectomized rats in suspended cages.

way a distinct difference in the activity of the experimental and the control animals but do not lend themselves at all readily to any sort of quantitative calculations. Absolute quantitation is out of the question because of over-lapping of individual tambour strokes. Runs were also made with a drum speed of 48 cm. per hour. While this prevented any considerable over-lapping of the individual movements on the drum, it did not obviate the difficulty of comparing them as to extent. It may be remarked in passing that the periodicity of activity reported by Richter (10) and others is quite apparent in the records of both the experimental and the control animals.

A second method of measurement employed was that of the revolving cage, arranged substantially as used by C. C. Stewart (11) as early as 1898 and later by Slonaker (9) and others. This consists of a cylindrical cage, the periphery of which is of hardware cloth, one-quarter inch mesh, and six inches wide, and the ends of galvanized sheet metal twelve inches in diameter. In communication with the cylinder is a small, rectangular feeding and sleeping cage $4 \times 4\frac{1}{2} \times 6$ inches in dimensions. This

consists of a metal drawer slipping into a three sided frame, the outer end of the drawer forming the rear end of the compartment. The front end is formed by a metal partition closing off one end of the revolving cylinder.

The sleeping compartment communicates with the cylinder by means of a hole two inches square in the partition. A 50 cc. beaker, held in one corner of the retiring cage by a metal strap, serves as a receptacle for soft food. A layer of oat-hulls or other suitable material a half to one inch thick covers the floor of the cage. The dimensions were planned to allow a mature rat to take a comfortable sleeping posture but to give the minimum space for activity. In a few cases screen top retiring cages were used. These proved to be less satisfactory, but did permit observations which showed that the rats expend relatively little energy by moving about in the retiring cages. It is believed on the basis of these observations that so large a proportion of the energy expenditure occurs in whirling the revolving cages that a record of the revolutions gives a reliable criterion of the vigor of the individual rats.

The revolutions are recorded by means of a "no. 6 rotary ratchet counter" supplied by the Veeder Manufacturing Company of Hartford. This is attached by a wire to an arm 1.5 cm. long extending at a right angle from the end of the shaft of the revolving cylinder. By this arrangement revolutions in either direction are summated. Partial revolutions are recorded as complete or not at all, depending upon the position of the cylinder at the time. Many observations have shown that the partial revolutions are so infrequent as compared with whole revolutions as to be negligible even though not compensated as just indicated. Readings were made at the end of each twenty-four hours and the hourly rate calculated for convenience in plotting. Figure 2, *A* and *B*, show typical curves of daily activity plotted at the average rate per hour. Since the cylinders are one foot in diameter each revolution corresponds approximately to a horizontal progression of 3.1416 feet. The actual distance run is less than this by a variable amount since the leap of the animal is across a chord of an arc. Another unavoidable variable is the "coasting" that certain animals indulge in. They run with unusual vigor for a period and then suddenly grasp the screen and revolve several times without effort. To what extent the preliminary burst of speed compensates for the "free-ride" would be hard to determine. It is apparent that the revolving cage method is only roughly quantitative. For investigations such as that herein reported in which the difference between the performance of experimental and control animals is very marked, the method is capable of yielding conclusive results with a relatively small number of animals. In cases, however, in which the differences are not marked, they would have to be determined on a more rigid statistical basis.

OBSERVATIONS. Twenty-four animals were decapsulated and seventeen were traumatized to serve as controls. Of the former, three died within an hour after the operation, two of these probably from an over-dose of amytal. Post-mortem examinations were made in all but two instances and no evidence of peritoneal or pulmonary infection was found. The remaining epinephrectomized animals survived for periods varying from three days up to three months or longer. They could not be distinguished by ordinary inspection from the controls, which were kept under exactly

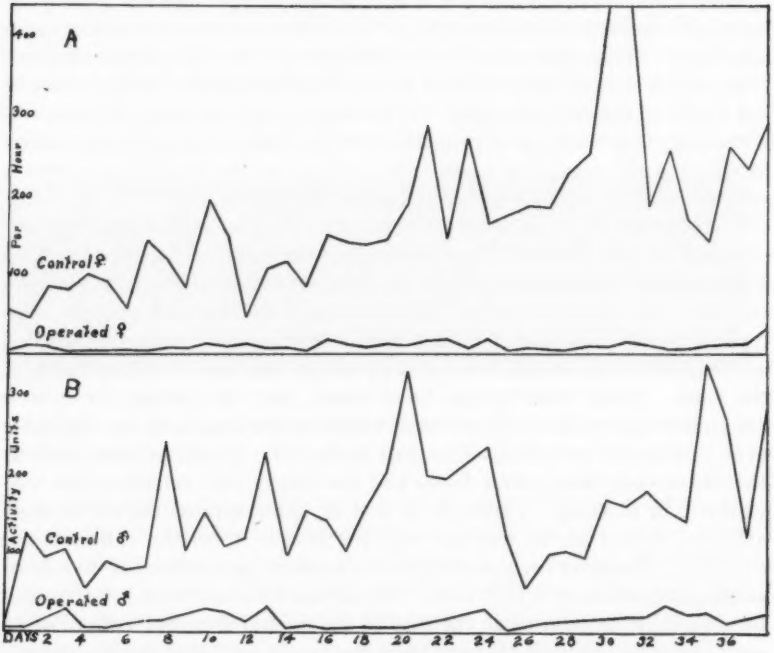

Fig. 2. Typical activity curves of 2 control and 2 epinephrectomized rats, showing the daily average number of cage revolutions per hour over a period of 38 days.

similar environmental conditions. All seemed alert and responded to the movements of the attendant and to the presence of food in the same way. Most of them were tried in the activity cages for periods varying from three days to seven weeks.

As previously stated, no difference between the general activity of experimental and control animals is apparent upon superficial examination, but study of a graph, such as figure 1, shows appreciably less activity in the case of the epinephrectomized animals, though the extent of individual

movements is often as great and the periodicity is quite similar to that of the normal. The typical curves of activity recorded by the revolving cages, reproduced as figure 2, show markedly restricted bodily activity following the removal of the adrenals.

The behavior of a single decapsulated animal, no. 3 of the series, is thought worthy of special mention. This animal was operated upon March 31 and placed in the suspended cage the night of April 3, the record showing less than the usual activity of the experimental animals, but otherwise being of typical character. On April 4 it worked again, showing considerably greater activity in the early part of the night but gradually decreasing until morning when it was found cold and apparently moribund. It was placed in the living quarters with other animals where it soon recovered and again worked on the night of April 12, when it manifested the typical degree of activity. The animal died April 14; examination failed to show any infection. Another somewhat similar instance is in apparent agreement with Erni's (12) finding that epinephrectomized rats induced to work to the point of extreme fatigue may succumb. Numerous observations seem to indicate that the revolving of the wheel serves as an effective stimulus to activity.

Attention may further be called to the several-day periodicity seen in case of both the experimental and the control animals. A limited number of records made in this laboratory to some extent agree with work subsequently published by Slonaker (13), showing a correlation between the periods of activity and the oestrous cycle. To a considerable extent the periods of augmented activity of the females such as shown in figure 2 occur during the periods of oestrum.

SUMMARY

1. The muscular activity of twenty-one epinephrectomized and sixteen traumatized control white rats was studied for periods of from three days to six weeks.

2. The animals from which both adrenals were removed appeared upon superficial observation to be normally alert and active but over a period of hours or days manifested markedly diminished activity.

3. The decapsulated animals survived the operation from three to one hundred and twenty days or longer (throughout the period of observation).

4. The experiments are interpreted as demonstrating chronic adynamia due to adrenal deficiency.

The writer wishes to acknowledge his obligation to Prof. R. G. Hoskins and Prof. A. M. Bleile for various helpful suggestions and assistance.

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THE ABSORPTION OF INSULIN FROM THE ALIMENTARY CANAL

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Since the discovery of insulin numerous attempts have been made to obtain the characteristic physiological effects of this hormone when administered orally. Nearly all such trials have resulted in total failure as measured by the inability to produce hypoglycemia in normal animals or to reduce the glycosuria of diabetic animals and patients. Some successes have been reported. Murlin and his co-workers (1) have secured evidence of activity when the material was introduced directly into the duodenum through a duodenal tube. The pancreatic extracts were given in 0.1 to 0.2 per cent hydrochloric acid with the purpose of preventing the action of trypsin. Positive responses were also elicited per os when the material was administered together with an acid buffer, the whole being enclosed in enteric coated capsules. Fisher (2) has shown that there is absorption of insulin when it together with dilute acid is injected directly into the gut. He found also that there was some absorption when the insulin was given by stomach tube to young puppies. Winter (3) reported that typical hypoglycemia and convulsions could be produced in rabbits when crude insulin in about 25 cc. of 20 per cent alcohol was given by stomach tube. He stated that as little as 1.5 to 2 times the subcutaneous dose was effective. Trials made in our laboratory (4) indicated that there was no absorption of insulin when given in alcohol by stomach tube to normal fasting rabbits. To be sure, a certain degree of hypoglycemia was produced but no more than occurred when alcohol of similar strength and amount was given. Since the publication of these two papers, Thatcher (5) has repeated these experiments and has concluded with us that there is no significant action when insulin is thus introduced. Murlin (1) has obtained effects with insulin administered in alcohol which seem to indicate that this menstruum somewhat favors the absorption of the hormone but good therapeutic effects have not been obtained by him. Salèn (6) found this mode of administration to be without noticeable effects upon normal rabbits and of no benefit in the treatment of diabetes. This author reports gratifying results when insulin was given orally with olive oil.

We record in this paper further experiments dealing with the absorption of insulin from the stomach when given in alcoholic solution. These negative experiments are followed by others in which typical insulin effects upon blood sugar and convulsions were produced in normal fasting rabbits. The potent fraction appears to be a very small part of the total insulin isolated from a given weight of pancreas. It is active in aqueous solution when given by stomach tube.

Our results show the importance of knowing the method of preparation of the material used in experiments dealing with the absorption of insulin from the gastro-intestinal tract. The insulin used in our previous study (4) was prepared by the original Collip method and was precipitated once and sometimes twice from alcohol of 96 per cent concentration. No acid was used in the extraction of the glands but the precipitations from alcohol were made at a pH of about 5.3. This insulin was relatively impure. The material used in the trials here reported was extracted in the same manner. Before distillation 7 to 10 cc. of concentrated sulfuric acid were added to each 4 gallons of 80 per cent alcoholic extract. After removal of the alcohol by distillation in vacuo and separation from fat, the insulin-containing material was salted out by the addition of 22 grams of NaCl per 100 cc. The salt precipitate was taken up in 80 per cent alcohol. In the first series of experiments presented below further purification was accomplished by precipitating with 2 volumes of ether followed by precipitations at the isoelectric point. This material was the regular insulin fraction used for the treatment of patients. In the second series, the material soluble in 80 per cent alcohol was precipitated by adding 4 volumes of absolute alcohol. The pH of these solutions was approximately 4.0 determined colorimetrically. The filtrates were concentrated to dryness in vacuo, dissolved in water and given by stomach tube. In the last series, the filtrates were made slightly alkaline with Na_2CO_3 or NaOH. At this reaction, pH 6.0 to 8.0, a precipitate was thrown down. The brown, gummy material was freed from alcohol, dissolved in water and administered to rabbits by stomach tube. It is to be noted particularly that the substance which is active when given by mouth is soluble in high concentration of alcohol and is found in the alcoholic filtrates from the regular insulin fraction. The glands of beeves, calves and sheep were used as the original source of material.

The preparations were assayed by the procedure outlined in another communication from this laboratory (7). The unit we use is the amount of insulin per kilogram of body weight which lowers the blood sugar of a normal fasting rabbit to the convulsive level after intravenous injection. The method of Folin and Wu was used for the determination of blood sugar. The rabbits used in the stomach tube experiments were fasted the preceding 24 hours.

It is perhaps well to emphasize certain precautions that should be observed in giving insulin by stomach tube. After the material has been given it should be washed down with 10 to 15 cc. of water and the tube should then be quickly removed. Before using again with another rabbit the tube should be thoroughly washed. These precautions are taken to obviate the possibility of some of the insulin being deposited in the respiratory tract. This is a real danger. We have observed typical insulin effects after a small amount of the hormone had been accidentally introduced into the lung. Any solution which injures the gastric mucosa will also make possible the absorption of insulin.

In table 1 are presented the changes produced in the blood sugar of rabbits when alcoholic solutions of relatively pure insulin are given by stomach tube. We endeavored in this series to approximate the concentration and amount of alcohol which Winter used. The results are very comparable with those reported in the previous communication from our laboratory (4). The decreases in blood sugar are of the same magnitude as those following the administration of alcohol alone. We had thought that the positive findings of Winter might have been due to a certain amount of injury to the gastric mucosa caused by the repeated doses of 20 per cent alcohol. The behavior of rabbit 23 gives no support to this conception. This animal was given insulin in 27 per cent alcohol four times. The last time this solution was given there was no change in the blood sugar level. Some of our insulin was subjected to dialysis. The blood sugar values for rabbits 19 and 7 show that neither the dialysate nor the residue in the collodion sac was absorbed. Our results, therefore, indicate that the regular insulin (isoelectric) preparations described exhibited no potency when given in alcohol to rabbits.

Table 2 shows the changes observed after the administration of a certain fraction of pancreatic extracts. This active fraction is found in the alcoholic filtrates from the regular insulin precipitate. It is soluble in alcohol of high concentration, up to at least 96 per cent and at a pH of about 4.0. Such filtrates were concentrated in vacuo until the alcohol was removed, leaving a brown, gummy mass. These residues were dissolved in water and given by stomach tube. In many cases the material was not entirely soluble in the volume of water used. The solutions were generally colloidal and often contained particles in suspension. Preparation 1, when given in 10 per cent alcohol, caused a typical hypoglycemic reaction. This response is to be compared with experiments using the same rabbit when alcoholic solutions of regular insulin were given, table 1. This was our first positive experiment. The significant drop in blood sugar encouraged us to determine if the material was active when introduced in water. Preparation 2 caused the typical hypoglycemia when given in 45 cc. of water. These residues contain rather large amounts of salt. In order to

rule out the effect of salt, 100 units of the regular isoelectric insulin in 45 cc. of 6 per cent NaCl solution were given to rabbit 6. No hypoglycemia resulted but instead there was a well-marked hyperglycemia. Preparations 7 and 12 when given in water caused hypoglycemia and convulsions. The convulsions were antidoted with glucose and the animals recovered in the usual manner. Rabbit 28 was given 600 units per kilogram of body weight of the regular insulin precipitated from 96 per cent alcohol without the

TABLE 1

Blood sugar of rabbits. Alcohol experiments

RABBIT		BLOOD SUGAR					INSULIN	MATERIAL GIVEN BY STOMACH TUBE
Number	Weight	Before	1 hour after	2 hours after	3 hours after	Change		
	kgm.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*per cent*	*kgm. units*	
4	2.49	105	118	111	105	0	50	20 cc. of 20 per cent alcohol Purified insulin, 0.006 mgm. N_2 per unit
8	1.64	133	105	111	105	−21	50	25 cc. of 27 per cent alcohol Purified insulin, 0.006 mgm. N_2 per unit
23	1.36	121	108	98	105	−19	50	30 cc. of 27 per cent alcohol Purified insulin. Intoxicated
8	1.64	125	118		111	−11	50	25 cc. of 27 per cent alcohol Purified insulin. Slightly intoxicated
23	1.36	167	125		105	−37	50	25 cc. of 27 per cent alcohol Purified insulin. Intoxicated
23	1.50	125	125	125	129	0	50	25 cc. of 27 per cent alcohol Purified insulin, 0.006 mgm. N_2 per unit
7	1.64	133	118	111	118	−17	66	25 cc. of 20 per cent alcohol Residue in sac after dialysis
19	1.45	111	100	100	111	−10	50	30 cc. of 20 per cent alcohol Material dialyzed through collodion

production of hypoglycemia. Negative results were also observed when the regular isoelectric product in water was introduced. Likewise, the crude aqueous solution from which the insulin is precipitated by salting failed to decrease the blood sugar.

The active material is also precipitated from 96 per cent alcohol at a pH of 6 to 8. The experiments in which this method was used are shown in table 3. On May 20, rabbit 35 was given some of the precipitate in solu-

tion of about 0.5 N alkali. The blood sugar decreased from 105 to 43 mgm. per 100 cc. one hour after the dose and convulsions occurred after 1.5 hours. We thought that this strength of alkali might have injured the gastric mucosa enough to allow absorption of the regular insulin. That such was not the case is shown by the fact that a large dose of isoelectric insulin given the following day was without effect upon the blood sugar. Rabbit 8 became hypoglycemic and had convulsions after receiving some of the precipitated material on May 29 . On June 2, the same preparation

TABLE 2

Blood sugar of rabbits. Fraction soluble in 96 per cent alcohol

RABBIT		UNITS GIVEN PER KILOGRAM	BLOOD SUGAR				CONVULSION	EQUIVALENT IN KILOGRAMS PANCREAS	NUMBER OF PREPARATION	MATERIAL GIVEN BY STOMACH TUBE
Number	Weight		Before	1 hour after	2 hours after	3 hours after				
	kgm.		*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*hours*			
23	1.46	78	105	57	69	114		28.8	1	45 cc. 10 per cent alcohol
20	3.46	19	108	55	55	95		28.8	2	45 cc. H_2O contained 6 per cent NaCl
24	1.13	790	87	91	33	85		14.0	7	45 cc. H_2O
2	1.58	506	121	55	45		2.5	12.8	7	40 cc. H_2O
31	1.08	833	105	47			1		12	50 cc. H_2O
					Controls					
28	2.09	600	125	138	129	125		4.2	363	Precipitate from 96 per cent alcohol in 25 cc. H_2O
35	2.10	620	111	129	111				262	Isoelectric product in 25 cc. H_2O
19	1.75	50	125	133	133	125			364	50 cc. crude aqueous solution before salting
6	1.50	100	133	182	200	182			260	Isoelectric product in 45 cc. of 6 per cent NaCl solution

was without effect when given to rabbit 18. This observation indicates that the fraction which is active when given in water is quite unstable, especially in aqueous solution. This is confirmed by our inability to find activity in this fraction in more than about 50 per cent of the trials. The remaining results show typical hypoglycemia produced by such preparations.

We wish to record other experiments in which insulin was injected directly into the small intestine of rabbits. One hundred units in 2.5 cc. water were injected into the small intestine of a rabbit without the produc-

tion of hypoglycemia. Approximately the same amount with 2 cc. of oleic acid was injected into the large bowel of another animal. No decrease in blood sugar resulted. These operations were done under novocaine anesthesia. Another rabbit was given 3 salol coated capsules containing 100 units of powdered insulin mixed with dried blood serum. A similarly treated $BaSO_4$ capsule was given at the same time. The fluoroscope showed this capsule remained in the stomach from 5 to 6 hours and was in the intestine at 9 hours and had begun to disintegrate. Symptoms of hypoglycemia were not observed.

The findings presented in this communication may be advantageously discussed at this point. Further experiments with the regular insulin

TABLE 3

Blood sugar of rabbits. Fraction precipitated from 96 per cent alcohol of pH 6 to 8

RABBIT		UNITS GIVEN PER KILOGRAM	BLOOD SUGAR				CONVULSION	EQUIVALENT IN KILOGRAMS PANCREAS	NUMBER OF PREPARATION	MATERIAL GIVEN BY STOMACH TUBE
Number	Weight		Before	1 hour after	2 hours after	3 hours after				
	mgm.		*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*hours*			
27	1.70	500	105	37			1		1 a	25 cc. H_2O. Reaction neutral
35	1.58	280	105	43			1.5		1 b	25 cc. 0.5 N NaOH Given May 20
35	1.58	625	111	129	111					Isoelectric product in 25 cc. H_2O. Given May 21
8	2.15	750	100	43			1.5	1.7	376	15 cc. H_2O. Given May 29
18	2.60	250	93	91	93			0.54	376	5 cc. H_2O. Given June 2
24	1.70	600	98	55	57			10.4	371	50 cc. H_2O
28	2.18	682	118	59	54			10.4	372	50 cc. H_2O

preparations show that there is no significant effect upon blood sugar when administered in alcohol. It is our belief that the regular isoelectric insulin as prepared by the methods used in our country is not potent when given by stomach tube. We have presented data which show that typical hypoglycemia and convulsions can be produced when a certain fraction of pancreatic extracts is given in water solution. The convulsions are successfully antidoted with glucose which indicates that the reaction is in fact due to insulin. This active fraction is soluble in alcohol of high concentration. In our work 96 per cent alcohol has been used. It is soluble in such alcohol when the reaction is acid but it is precipitated at a pH of 6 to 8 determined colorimetrically. The best yield obtained represents an equivalent of about 1.7 kgm. of pancreas which produced a

convulsion in a rabbit weighing 2.6 kgm. This small yield affords ground for speculation. It is well known that the total amount of insulin obtained from the pancreas of an animal is more than enough to render it hypoglycemic and to produce convulsions. One may conceive of an active insulin and an inactive or pre-insulin form being present in the gland. The ordinary methods of extraction may extract the latter form and a part of the first. Again, alcohol may not be a good extractive for this small fraction. Another possibility is that the action of alcohol produces a chemical change in a very small fraction of the regular isoelectric insulin. In this connection one naturally thinks of the active and inactive forms of thyroxin and adrenalin. Those who believe that insulin is a definite chemical entity of simpler constitution than the polypeptides will perhaps favor the view that the active portion contains some of this substance. One must also consider the possibility that the material may only stimulate the pancreas to increased activity. The blood sugar curves and the recovery from convulsions by the administration of glucose render the latter hypothesis quite unlikely. We suggest that the positive results of Winter, Murlin and others were probably secured by the presence of this active material in their extracts. The investigation is being furthered along the lines outlined above.

SUMMARY

Further experiments are reported which show that there is no significant effect upon blood sugar when regular insulin in alcohol is given by stomach tube to rabbits.

A small fraction of pancreatic extracts produces typical insulin hypoglycemia and convulsions when given per os in water solution. The material is soluble in acid alcohol of at least 96 per cent concentration and is precipitated from this liquid at a slightly alkaline reaction.

It is a pleasure to acknowledge the assistance of Mr. John Berger and Mr. Melville Sahyun.

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STUDIES ON THE NERVOUS REGULATION OF PROGRESSION IN MAMMALS

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It is a well-established fact that the nervous mechanism in the lumbar and sacral regions of the cord is in itself sufficient to produce the rhythmical sequence of flexion and extension in the hind legs which constitutes the reflex step. Goltz, Freusberg (5) and later Phillipson (9) showed that in the late spinal dog, reflex stepping was elicited in the hind legs in response to various stimuli applied to the legs and feet. These movements also occurred spontaneously when the animal was supported in a vertical position, the hind legs hanging freely. In these experiments the stimulus for eliciting the movements was thought to be stretching of the skin.

Sherrington (12), (13) proved that, in the decapitate cat, stepping was prominent in the hind legs in response to nociceptive stimuli applied to various regions of the body, i.e., clip on the perineum, foot and other regions of the skin; also to electrical stimuli applied to afferent nerves in the legs. He further showed that it was much more difficult to elicit reflex stepping in the forelegs than it was in the hind legs. Miller (8) observed that nociceptive stimuli applied to the pads of the fore paws gave rise to reflex stepping in the hind legs of the decapitate cat but not in the forelegs. All concluded that the rhythmical response shown in the reflex step was referable to the nervous mechanism in the cord concerned with the reflexes.

Graham Brown (2), (3) proved that the rhythmical sequence of flexion and extension occurred, in cats, after the dorsal roots of the nerves supplying the legs had been cut. He suggested that the rhythmical sequence of flexion and extension was due to phasic changes in the nerve centers of the cord, and was not initiated by sensory or proprioceptive impulses as suggested by Sherrington. He thought that these rhythmical movements were due to a balance between equal and opposite states producing flexion and extension; also that the proprioceptive impulses in the normal animal may act by grading the individual component movements to variations in the environment.

The reflex movements of the spinal step do not constitute the complex movements of the limbs observed in the walk, run or gallop, where there is

a definite coördinated sequence of events in the hind and forelegs. These coördinated movements in the hind and forelegs have, to the best of my knowledge, never been described in decapitate preparations. It therefore seems probable that the nervous mechanism which functions in making possible the coördination in locomotion is so arranged that removal of the portion of the cerebro-spinal axis cephalad to the spinal cord eliminates the coördinated movements of progression. Thiele (16) stimulated the mesencephalic tracts in decerebrate cats, electrically, and obtained movement similar to those of progression. He concluded that there was a coördinating "center" for progression which was located in the thalamus. The tracts which function in giving the coördinated movements were considered to be the rubrospinal, tectospinal, thalamospinal and thalanobulbar.

The present work was undertaken in order to determine the extent of the nervous mechanism in the cerebro-spinal axis which renders possible the coördinated movements of progression in the rabbit, cat and dog. In the cat and dog the movements observed in the hind and forelegs during the act of walking and trotting are as follows: The hind and forelegs show an alternate rhythmical flexion and extension, the right hind leg being carried forward simultaneously with the left foreleg while the left hind leg is carried forward with the right foreleg. In the rabbit, during the act of progression, there is also a definite sequence of events. The forelegs show an alternate rhythmical flexion and extension while the hind legs show a synchronous bilateral flexion and extension. In this work, the movements described above will be referred to as "coördinated movements of progression" for the cat, dog and rabbit respectively.

In preliminary experiments it was found that in decerebrate rabbits, the section through the brain stem being slightly cephalad to the superior colliculi dorsally and slightly caudad to the mammillary bodies ventrally, stimulation of the dorsal cutaneous nerves in the cervical, thoracic, lumbar and sacral regions of the body, resulted in powerful coördinated movements of progression. The conclusion was then reached that the nervous mechanism which functions in making possible any coördinated movements of progression in the rabbit was so arranged that removal of the brain cephalad to this level did not interfere with its normal activity. Attempts were then made, by methods to be described, to eliminate these movements in all four legs simultaneously, the forelegs alone and the hind legs alone by producing lesions in the midbrain, cerebellum and spinal cord.

RABBITS. *Methods. 1. Coördinated movements of progression in all four legs of the rabbit.* Domestic rabbits were used in these experiments. The animals were anesthetized with ether, tracheotomy was done and the dorsal cutaneous nerves from the 4th, 5th and 6th thoracic nerves were isolated and prepared for stimulation. Early in this work it was observed that the results were identical when the stimulated nerve was in the cervi-

cal, thoracic, lumbar or sacral regions, hence in the majority of the experiments the dorsal cutaneous nerves from the 4th, 5th and 6th thoracics on both sides were stimulated. These nerves were chosen because they were easily isolated and when the electrodes were applied they did not interfere with the movements under observation in the legs. Stimulation of afferent nerves has advantages over the method of stimulation of the brain-stem and mesencephalic tracts directly, in that one is dealing with the afferent portion of intact reflex arcs. These afferent nerves normally carry impulses which, no doubt, have a marked influence on the behavior of the animal as a whole. In other words, this method of using the coördinated complex reflex is more refined.

The rabbits were decerebrated in the following manner. Both carotids were tied, the skull trephined and the top of the cranium removed, hemorrhage from the bone was controlled with wax. The dura was opened and the cerebral hemispheres were pulled forward so as to expose the mid-brain. A clean cut was made through the brain-stem at a level slightly cephalad to the superior colliculi dorsally and slightly caudad to the mammillary bodies ventrally (fig. 1). All the brain tissue cephalad to the section was removed. The hemorrhage from the vertebral arteries was controlled by pressure applied behind the wings of the atlas and by packing the cranial cavity with dry absorbent cotton, care being taken to have no pressure on the mid-brain. The wound was now closed and after about five minutes the pressure was removed from the vertebral arteries. The operations were all performed on a heated table and the temperature of the animals was kept at 37°C. throughout the experiments.

Reflex movements were observed in response to mechanical (pinch) and electrical stimuli. The electrical stimuli were faradic currents from a "Kershaw" induction coil supplied by an Edison potash battery. The faradic currents were applied to the dorsal cutaneous nerves by means of bipolar platinum electrodes. The strength of the stimulus was kept constant, having a current of 0.3 amp. in the primary and a secondary distance of 120 mm. This strength of stimulus was sufficient to elicit the coördinated movements of progression when applied to the dorsal cutaneous nerves and there were no movements of any kind produced by escape to neighboring tissues. After the coördinated movements of progression were eliminated, the strength of the stimuli was increased in order to see if coördinated movements could still be elicited by the stronger stimuli.

Following the observations, the decerebration having been done at the level described above, the animal was again anesthetized and a section made through the mid-brain at a level caudad to the primary section. The animal was allowed to recover and the reactions studied as before.

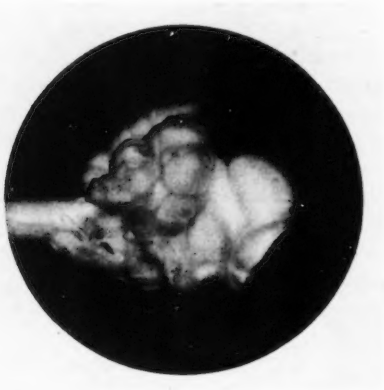

Fig. 1. Rabbit brain, showing level of section through brain-stem as described in text.

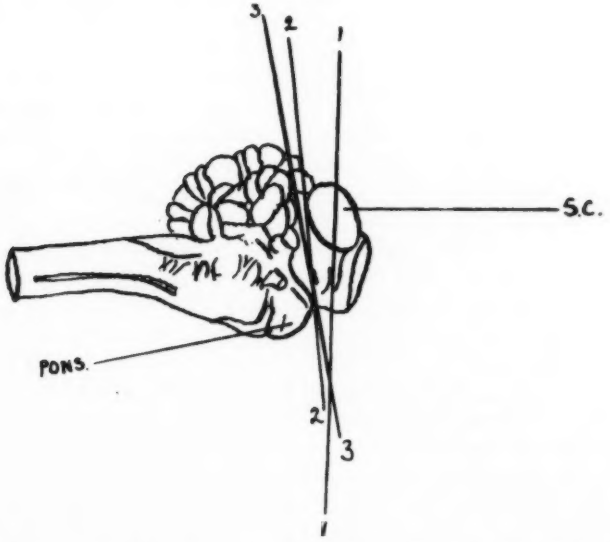

Fig. 2. Diagram to show the levels of transection through the mid-brain, *1-1* level 1, *2-2* level 2, *3-3* level 3, described in text. Level 3 indicates the most caudad level at which coördinated movements of progression could be elicited in all four legs. *sc.*—superior colliculi, *pons*,—pons.

This procedure was continued until the coördinated movements of progression were eliminated. The most caudal level at which the coördinated movements of progression were still present was determined and also the level at which the movements were completely eliminated. The nervous mechanisms which make possible the coördinated movements of progression were considered to be so arranged that the portion of the brain-stem between these two levels must be intact, in order that they may function normally. In the majority of the experiments the coördinated movements of progression were eliminated by the above procedure, while in others the primary section was made at the level which eliminated the coördinated movements. Invariably the animals in which the initial section was made at the caudal level described above, showed no coördinated movements of progression.

2. Forelegs studied alone. The spinal cord was completely transected at the level of the last thoracic vertebra. The dorsal cutaneous nerves from the 4th, 5th and 6th thoracic nerves were isolated and prepared for electrical stimulation; also some of the dorsal cutaneous nerves in the lumbar and sacral regions were prepared. The decerebration was done as described above and the rhythmical alternate movements of progression in the forelegs were eliminated by similar methods. Reflex movements in the hind legs were observed in these preparations.

3. Hind legs studied alone. Double ligatures were tied around the entire brachial plexuses on both sides and the nerves cut between the ligatures. This operation completely eliminated all reflexes in the forelegs. The dorsal cutaneous nerves were prepared for stimulation and the decerebration done as previously described. Synchronous bilateral movements in the hind legs were eliminated.

4. Decapitate rabbits. The animals were decapitated by the method used by Sherrington (14) for the cat. An effort was made to elicit movements of progression by using various types of stimuli.

5. Late spinal rabbits. The spinal cord was transected under aseptic conditions at the level of the last thoracic vertebra. After a period of several weeks had passed the animal was prepared as described above and the coördinated movements in the forelegs studied and eliminated.

6. Rabbits with cerebellum removed. Animals were anesthetized, tracheotomy was done and the dorsal cutaneous nerves prepared for stimulation. The cerebellum was completely removed and the animal decerebrated immediately. The coördinated movements of progression were studied in all four legs and eliminated as before.

7. Rabbits with thalamus intact. Decerebration was done at a level through the cephalic end of the thalamus The reactions of the animals were then observed.

Observations. 1. All four legs studied together. When the decerebration was done at a level slightly cephalad to the superior colliculi dorsally and slightly caudad to the mammillary bodies ventrally the following results were obtained. The rigidity was very slight and the shock resulting from the decerebration was at first marked, but soon disappeared. About five minutes after the decerebration, a strong mechanical stimulus applied to the toes of the hind feet resulted in a slight ipsilateral flexion of the leg. The reflex was, at first, very sluggish but became more brisk and after about ten minutes it was accompanied by crossed extension in the contralateral leg. About this time the forelegs showed the flexion reflex in response to mechanical stimuli applied to the toes of the fore feet. The reflex was at first sluggish but soon became quite brisk and was accompanied by adduction in both forelegs and extension in the contralateral leg. These observations are in agreement with those made by others in that they show that the reflexes in the hind legs return more quickly after decerebration than they do in the forelegs and hence it is considered that the cord mechanisms in the lumbar and sacral regions are not so profoundly affected by the shock resulting from the removal of the higher centers in the brain, as are those in the cervical and upper thoracic regions.

About fifteen to twenty minutes after the decerebration, faradic currents of the strength indicated above, applied to the dorsal cutaneous nerves, elicited coördinated movements of progression. These movements persisted for a considerable time after the stimulus was removed. The rabbits were very sensitive to the faradic currents, an instantaneous application to the nerves being sufficient to call forth the movements of progression.

When the section through the mid-brain passed through the superior colliculi dorsally and about 2 mm. cephalad to the anterior border of the pons ventrally, figure 2, level 1, or caudad to either the superior colliculi or the inferior colliculi dorsally and just cephalad to the anterior border of the pons ventrally, figure 2, levels 2 and 3, the decerebrate rigidity was very intense and the shock resulting from the operation was profound. As above the reflexes returned earlier in the hind legs than in the forelegs. If the initial section was made at the level shown in figure 1, and subsequently sections were made at levels indicated in figure 2, the rigidity increased but the shock resulting from the later sections was very slight. The reflexes in the legs and the coördinated movements of progression were present almost immediately after the later sections were made. These results are similar to those described by Sherrington (15) where transection of the cord, at a level caudad to a primary section, gave no indication of shock in those parts innervated by that portion of the cord caudad to the second section.

Stimulation of the dorsal cutaneous nerves with faradic currents, the decerebration having been done at levels indicated in figure 2, resulted in very rapid coördinated movements of progression. The movements were not elicited as easily as when the level of transection was that shown in figure 1, and they ceased while the stimulus was still being applied. The movements were just as rapid as when the section was at the higher level, figure 1, and appeared to break through the rigidity which was very marked in all the legs. That the rigidity influenced the movements slightly was shown by modifications in the foreleg movements. The flexion in the forelegs was not as complete but the alternate rhythmical character was prominent. The movements in the hind legs did not appear to be affected by the rigidity and the bilateral synchronous movements showed in their true form. Pinch clips applied to the toes and perineum gave results identical to those described above, resulting from the stimulation of the dorsal cutaneous nerves with the faradic currents.

When the section was made through the brain-stem at a level just caudad to the inferior colliculi dorsally and about 1 mm. caudad to the cephalic border of the pons ventrally, (fig. 3, and fig. 4, level 1) the results obtained on application of mechanical and electrical stimuli were different. Electrical stimuli applied to the dorsal cutaneous nerves, and mechanical stimuli applied to the toes failed to elicit the alternate rhythmical movements in the forelegs. The hind legs, on the other hand, gave powerful synchronous bilateral movements as observed in the coördinated movements of progression. Stronger faradic currents applied to the dorsal cutaneous nerves failed to elicit the rhythmical movements in the forelegs but there were muscular changes due to escape of current to the neighboring tissues. The rigidity in all the legs was markedly increased following the application of the stimuli. From these results it is evident that the bilateral synchronous movements of the hind legs are not initiated by the alternate rhythmical movements in the forelegs. Furthermore, it is evident that the nervous mechanism which functions in making possible the coördinated movements of progression is so arranged that removal of the cephalic portion of the pontine region results in the elimination of the movements in the forelegs but does not affect the synchronous bilateral movements in the hind legs.

When the sections passed through the brain-stem caudad to the inferior colliculi dorsally and at the caudal level of the pons ventrally, figure 5, and figure 4, level 2, faradic currents applied to the dorsal cutaneous nerves and strong mechanical stimuli applied to the toes, perineum and skin failed to elicit the coördinated movements of progression in forelegs and hind legs. Stronger electrical stimuli (sec. dist. 120–0) applied to the dorsal cutaneous nerves failed to give the coördinated movements of progression. Strong mechanical and electrical stimuli applied to the hind feet gave

ipsilateral flexion and crossed extension in the hind legs. The same stimuli applied to the fore feet gave ipsilateral flexion and crossed extension in the forelegs. At no time during the experiment, which lasted for several hours, was there any indication of the coörd nated movements of progression.

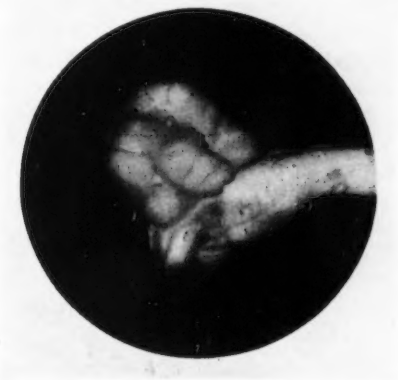

Fig. 3. Rabbit brain, showing level of transection at which the alternate rhythmical movements of the forelegs were eliminated.

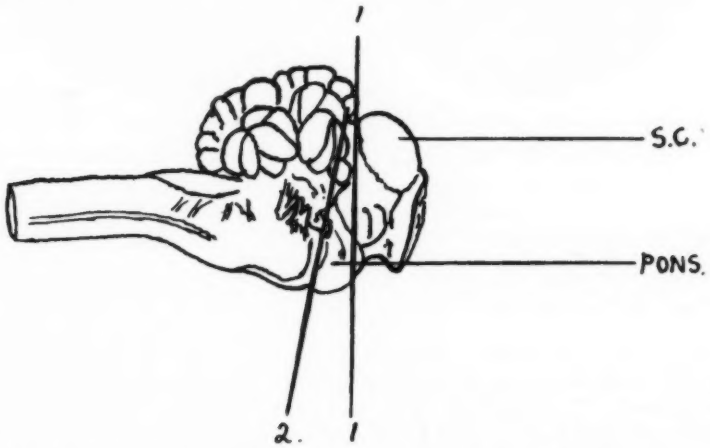

Fig. 4. Diagram of rabbit brain showing level *1-1*, at which the alternate rhythmical movements of the forelegs were eliminated; level *2-2*, at which the synchronous bilateral movements of the hind legs were eliminated and the coördinated movements of progression in all four legs when the primary section was made at this level. *sc.*—superior colliculi, *pons*—pons.

The rigidity, in these animals, was still present but was not so intense as when the section was made at a higher level in the mid-brain.

Medial longitudinal sections extending from the cephalic end of the superior colliculi through the mid-brain to the caudal border of the pons, in rabbits decerebrated at the level shown in figure 1, resulted in profound shock and completely eliminated the coördinated movements of progression. It is evident, then, that this coördinating mechanism depends, for its normal functioning, on the integrated action of the neurons located on both sides of the mid line in the region of the pons.

2. Rabbits with cerebellum removed. Rabbits, in which the cerebellum had been entirely removed, were decerebrated at the level shown in figure 1. The rigidity following the removal of the cerebellum was quite intense

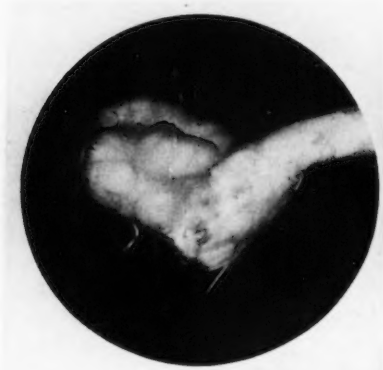

Fig. 5

Fig. 6

Fig. 5. Rabbit brain, showing level of transection through the pontine region at which the coördinated movements of progression were eliminated when the primary section was made at this level and also the level at which the synchronous bilateral movements of the hind legs were eliminated.

Fig. 6. Cat brain, showing the posterior level through the thalmus at which section could be made and still obtain coördinated movements of progression.

but rapidly became less. The rigidity following the decerebration was more intense than that which was observed in animals decerebrated at the same level, the cerebellum being intact. Weak faradic currents applied to the dorsal cutaneous nerves and mechanical stimuli applied to the toes and perineum resulted in coördinated movements of progression. The movements in the forelegs were somewhat different to those previously described. When the stimulus was applied to the dorsal cutaneous nerves the forelegs were drawn into a semi-flexed position while they performed the alternate rhythmical movements. The movements ceased while the

stimulus was being applied to the nerves, the legs gradually extending with marked rigidity.

3. Forelegs studied alone. The spinal cord was completely transected at the level of the last thoracic vertebra either at the time of the experiment or a few days before in order that the effects of spinal shock might pass off. The results obtained were identical regardless of the time of spinal transection. The animal was now decerebrated and the alternate rhythmical movements in the forelegs were eliminated by the methods described above.

Mechanical stimuli applied to the hind feet and the perineum resulted in ipsilateral flexion and crossed extension in the hind legs but at no time was there any indication of the synchronous bilateral movements in the hind legs. Weak and strong faradic currents applied to the dorsal cutaneous nerves in the lumbar and sacral regions gave slight reflex muscular movements in the hind legs but no synchronous bilateral, or alternate rhythmical movements in the hind legs. As would be expected, stimuli applied to parts caudad to the transection of the cord, gave no movements of the muscles innervated by the cord cephalad to the section.

The movements in the forelegs, in response to mechanical stimuli applied to the toes of the fore feet and to electrical stimuli applied to the dorsal cutaneous nerves in the thoracic region were identical with those described for the forelegs in animals with the spinal cord intact. Sections through the brain-stem just caudad to the inferior colliculi dorsally and about 1 mm. caudad to the cephalic border of the pons ventrally, figure 3, and level 1, figure 4, eliminated the alternate rhythmical movements in the forelegs. Following this section, no stimulus, however strong, was able to elicit these movements. This level, at which the alternate rhythmical movements of the forelegs were eliminated, corresponded exactly with the level at which the movements in the forelegs were eliminated when the spinal cord was intact.

4. Hind legs studied alone. The forelegs were immobilized by a bilateral section of the entire brachial plexus on both sides. The decerebration was done immediately in the usual manner and the responses of the hind legs to electrical and mechanical stimuli studied.

Decerebration at a level cephalad to the superior colliculi dorsally and just caudad to the mammillary bodies ventrally, figure 1, caused considerable shock and there was a slight rigidity in the hind legs. After about fifteen minutes, weak faradic currents applied to the dorsal cutaneous nerves in the thoracic region elicited the synchronous bilateral movements in the hind legs which were identical with those described for the hind legs during the normal act of progression. The movements were very rapid and persisted for a short time after the removal of the stimulus. Mechanical stimuli applied to the perineum and toes of the hind feet gave similar results.

Sections through the mid-brain at levels 1, 2 and 3, figure 2, and level 1, figure 4, produced a very marked rigidity in the hind legs. Mechanical and electrical stimuli applied as described above elicited synchronous bilateral movements in the hind legs. The movements appeared to break through the rigidity and were not as rapid as when the section was made at the higher level. Frequently the movements ceased while the stimulus was being applied but in a large percentage of cases they ceased coincidently with the withdrawal of the stimulus.

When the section passed through the brain-stem just caudad to the inferior colliculi dorsally and the caudal border of the pons ventrally, figure 5 and level 3, figure 4, the rigidity was less than when the sections were made at the higher levels in the brain-stem. Mechanical and electrical stimuli, applied as described above, failed to elicit the synchronous bilateral movements in the hind legs. Increase in the strength of the stimulus had no effect. At no time during the experiment, which lasted for several hours, did the animal show any signs of synchronous bilateral movements in the hind legs. It was apparent then that the sections at this level eliminated the movements and the conclusion reached that the nervous mechanism which functions in making possible the synchronous bilateral movements in the hind legs of the rabbit is so arranged that the portion of the brain-stem which lies in the pontine region must be intact to insure the synchronous bilateral movements of progression in the hind legs. This portion of the brain-stem lies caudad to the portion whose removal eliminated the alternate rhythmical movements in the forelegs.

5. Late spinal rabbits. In late spinal rabbits (10 days after spinal transection), the decerebration having been done as described above, the responses in the forelegs were the same and the alternate rhythmical movements were eliminated at the same level. Weak and strong faradic currents applied to the dorsal cutaneous nerves in the lumbar and sacral regions failed to give the synchronous bilateral movements in the hind legs. Mechanical stimuli applied to the toes of the hind feet gave ipsilateral flexion and crossed extension in the hind legs. *Four weeks after the spinal transection,* the rhythmical "mark-time" reflex as described by Freusberg (5) and Phillipson (9), in the late spinal dog, and by Sherrington (12), (13) in the cat, was observed when the animal was held in a vertical position with the hind legs pendant. The "mark-time" reflex was also produced by stimulation of the toes of the hind feet with mechanical stimuli. There was at no time any indication of the synchronous bilateral movements in the hind legs and it seems reasonable to conclude that the nervous mechanism in the lumbar and sacral regions of the cord of the rabbit, while in itself sufficient to produce the alternate rhythmical movements which resemble the step, is not sufficient to produce the synchronous bilateral movements as observed in the hind legs during the normal act of progression.

6. Decapitate rabbits. The rabbits were decapitated by the methods used by Sherrington (14) for the cat. The reflex responses of the animals were studied, using mechanical and electrical stimuli applied to the feet and perineum, and weak and strong faradic currents applied to the dorsal cutaneous nerves in the thoracic, lumbar and sacral regions.

Strong mechanical and electrical stimuli applied to the hind feet produced ipsilateral flexion and crossed extension in the hindlegs. The same stimuli applied to the fore feet produced ipsilateral flexion and crossed extension in the forelegs. Stimulation of the dorsal cutaneous nerves in the thoracic region resulted in some slight muscular movements in the forelegs but none in the hind legs, also stimulation of the dorsal cutaneous nerves in the lumbar and sacral regions resulted in slight muscular movements in the hind legs but none in the forelegs. There was absolutely no indication of coördinated movements of progression in such animals throughout the experiments, which lasted several hours. Reflex stepping as described by Sherrington (12), (13) and by Miller (8) for the cat, was not observed in the decapitate rabbit.

7. Rabbits with thalamus intact. Rabbits, in which the decerebration was done through the cephalic end of the thalamus, showed many normal reactions. In about twenty minutes the shock due to the operation had passed off and the animal made efforts to rise. They assumed a normal sitting posture but in only a few instances did they show spontaneous efforts to run or hop. When the animals were stimulated by pinching the toes, tail or perineum, they responded by hopping about the room. They maintained a perfect equilibrium during this act of locomotion but were unable to avoid objects placed in their pathway. When an obstacle was encountered the movements of progression generally ceased and the animal assumed a sitting posture facing the object.

8. Observation on shock resulting from sections through the thalamus. The primary section through the cephalic end of the thalamus was attended with considerable shock. Shock, from succeeding sections through the thalamus caudad to the first, was not so profound. After the primary section the reflexes in the legs began to appear after about ten minutes, and became very brisk about twenty minutes after the operation when running movements were observed. After sections through the thalamus caudad to the primary section the reflexes in the legs began to appear after about two minutes and the coördinated movements of progression were observed after seven to ten minutes. These observations on shock are different from those to be described for the cat and dog after similar operations.

Cats. *Methods.* The animals were anesthetized with ether. Tracheotomy was done and both common carotids tied. The animal was placed in a prone position, the head being secured in a suitable holder.

The skull was trephined and the dorsal part of the cranium was removed, hemorrhage from the bone being controlled with wax. The animals were decerebrated by pulling the cerebral hemispheres forward and making a clean cut through the cephalic end of the thalamus which reached the ventral surface of the brain at the cephalic end of the infundibulum. (Fig. 6, and level 1, fig. 7, and fig. 8.) The cerebral hemispheres and the portion of the brain-stem cephalad to the section were removed from the cranial cavity which was then packed with absorbent cotton. Hemorrhage from the vertebral arteries was further controlled by pressure applied to the sides of the neck just behind the wings of the atlas. It was difficult in some of the experiments to remove all of the corpus striatum but in view of the work of Wilson (17) it is considered that the corpus striatum is not the portion of the brain which functions in making possible the coördinated movements of progression.

The animals were allowed to recover from the shock of the operation and their reactions to various stimuli observed. The stimuli used were the same as those used for the rabbit, the mechanical stimuli being applied to the feet and perineum and the electrical stimuli being applied to the dorsal cutaneous nerves in the thoracic region and to the perineum. The animals were again anesthetized and a section made through the thalamus at a level caudad to the primary section. They were again allowed to recover and the responses to electrical and mechanical stimuli were noted. This process was continued until the coördinated movements of progression were eliminated. Observations on shock and rigidity were noted and also the effect of medial longitudinal sections through the thalamus.

Observations. Shock resulting from sections through the cephalic part of the thalamus was very marked. There was slight indication of the ipsilateral flexion reflex in the hind legs after about ten minutes, and shortly after they were indicated in the forelegs. The flexion reflex in the fore- and hind legs gradually became more brisk and after 20 to 30 minutes they were very prominent. Spontaneous movements were observed in the animals after about 30 minutes. They made efforts to rise but were unsuccessful, equilibration was greatly disturbed and the legs were unable to support the body and showed no definite coördinated movements. About 45 minutes after the decerebration, faradic currents, of the strength indicated above, applied to the dorsal cutaneous nerves in the thoracic region, elicited coördinated movements of progression. The nerves were stimulated and the animal was allowed to walk on the floor. Equilibration was greatly improved and the animal could stand and walk with difficulty, the legs seeming to be unable to support the body, the animal moving forward in a crouching attitude. After about an hour and a half the animal could walk quite normally, the body was well supported and the movements of progression were well coördinated. These animals reacted well.

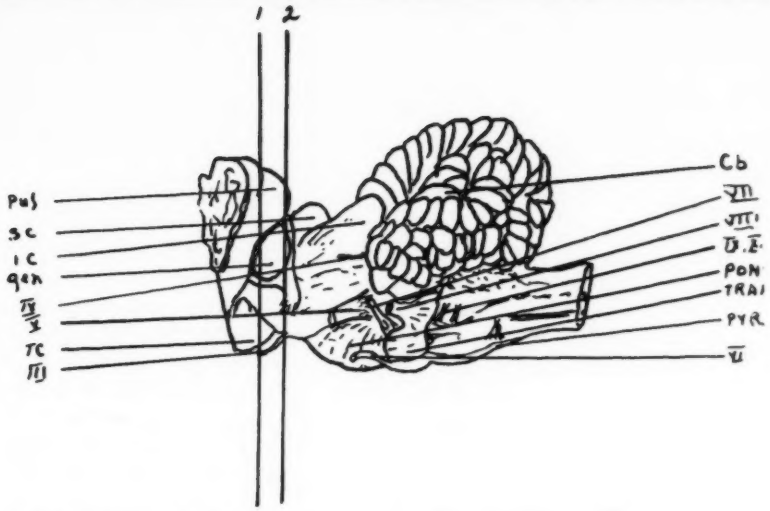

Fig. 7. Diagram of cat brain; *pul.*—pulvinar, *sc.*—superior colliculus, *ic.*—inferior colliculus, *gen.*—medial geniculate body, *tc.*—tuber cinereum, *cb.*—cerebellum, *trap.*—trapezium, *pyr.*—pyramid, *pons*, III, IV, V, VI, VII, VIII, IX, X, cranial nerves, *2.*—level at which movements of progression were eliminated in all four legs and at which the alternate rhythmical movements were eliminated in the hind legs. *1*,—level at which the alternate rhythmical movements of the forelegs were eliminated.

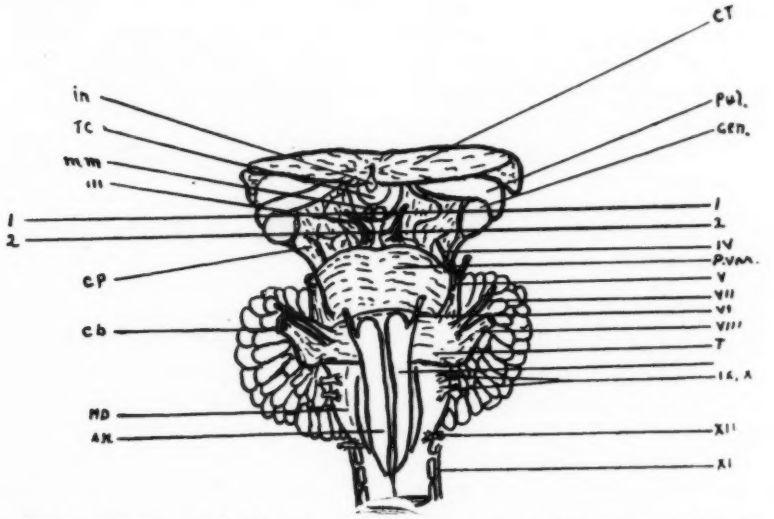

Fig. 8. Diagram cat brain, ventral surface; *in.*—infundibulum, *tc.*—tuber cinereum, *mm.*—mammillary bodies, *ct.*—cross section through the thalamus, being the most caudal level at which sections could be made and still have coördinated movements of progression in all four legs. *pul.*—pulvinar, *gen.*—medial geniculate body, *p. var.*—pons varoli, *t.*—trapezium, *md.*—medulla, *an.*—pyramid, III–XII cranial nerves. *1-1*, ventral level of section which eliminated the alternate rhythmical movements of the forelegs. *2-2*, ventral level of section which eliminated the coördinated movements of progression in all four legs, and at which the alternate rhythmical movements of the hind legs were eliminated.

They responded to nociceptive stimuli by progression but the movements lacked the guiding influence of the cerebral centers, the animals wandering about aimlessly. They were unable to avoid objects placed in their pathway and when one was encountered the animal acted like an automaton, occupying a position facing the object the head being pressed against it while the legs performed the normal walking movements.

When the decerebration was done at the level described above, figure 6, and the animal suspended, nociceptive stimuli applied to the pads of the feet and perineum and faradic currents (0.3 amp. primary and sec. dist. 120 mm.) to the dorsal cutaneous nerves, produced coördinated movements of progression. The movements persisted for a considerable time after the removal of the stimuli. When the faradic currents were applied to the nerves over an extended period of time (30 to 60 seconds) the hind legs developed a synchronous bilateral movement similar to those described for the rabbit. These synchronous bilateral movements gradually faded into the alternate rhythmical movements which persisted for some time after the removal of the stimuli. The movements in the forelegs were always of the alternate rhythmical type regardless of the time of application of the stimulus.

When the section passed through the caudal third of the thalamus dorsally and through the cephalic end of the mammillary bodies ventrally, figure 9, level 1 figure 8, and level 2 figure 7, the responses of the animal were different. The shock produced was profound, the rigidity was intense and at no time during the experiment was there any indication of spontaneous movements. Reflexes in the legs began to appear after about ten minutes and after about thirty minutes they were quite brisk. Mechanical stimuli applied to the pads of the feet and faradic currents applied to the dorsal cutaneous nerves failed to elicit the alternate rhythmical movements in the forelegs throughout the experiments. The same stimuli, however, produced the alternate rhythmical movements in the hind legs after about thirty minutes. If the stimulus was prolonged synchronous bilateral movements developed in the hind legs which gradually faded into the movements of the alternate rhythmical type. The movements in the hind legs did not persist after the removal of the stimulus as was observed when the section was made at the higher level. After the stimulus was removed the rigidity in the hind and forelegs became very intense.

These results indicate that the coördinating mechanism for progression in the cat is so arranged that removal of the middle third and the cephalic portion of the caudal third of the thalamus results in the elimination of the coördinated movements in the forelegs while the movements in the hind legs are unaffected.

Sections which passed through the brain-stem slightly cephalad to the superior colliculi dorsally and just cephalad to the roots of the oculomotor

nerves ventrally, figure 10, level 3 figure 7, and level 2 figure 8, resulted in a very marked rigidity in all the legs. Shock resulting from these sections was just as profound as when the sections were made at higher levels through the thalamus. After this section, no stimulus, however strong, elicited the alternate rhythmical movements in the hind legs. If the primary section was made at this level no type of stimulus elicited the coördinated movements of progression.

It is evident then that, in the cat, the coördinating mechanism which makes possible the movements of progression is so arranged that removal of the caudal two-thirds of the thalamus and a small portion of the cephalic end of the mid-brain, figure 7, results in the elimination of the coördinated movements of progression.

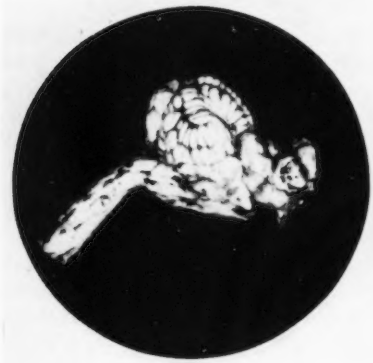

Fig. 9

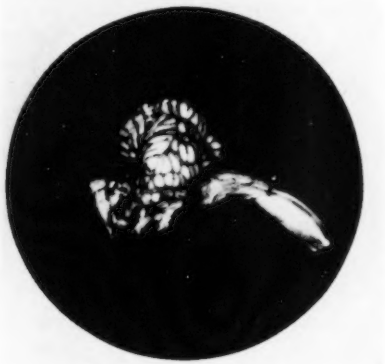

Fig. 10

Fig. 9. Cat brain, showing level, through the thalamus, at which sections eliminated the alternate rhythmical movements of the forelegs.

Fig. 10. Cat brain, showing level through the brain-stem at which sections eliminated the coördinated movements of progression in the fore- and hind legs and at which the alternate rhythmical movements of the hind legs were eliminated.

Medial longitudinal sections through the thalamus extending slightly into the mid-brain resulted in marked rigidity in all the legs, the shock being very profound. The coördinated movements of progression were entirely eliminated but ipsilateral flexion and crossed extension reflexes in the hind and forelegs were readily elicited after the shock had passed off. It would appear then that, as in the rabbit, the normal act of progression in the cat depends on the integrated action of the nervous mechanisms located on either side of the mid-line.

KITTENS. *Methods.* The kittens used in these experiments were four weeks old and were able to walk and run. The methods were identical with those used for the adult cat.

Observations. When the level of decerebration was through the cephalic end of the thalamus the shock and rigidity were very slight. Coördinated movements of progression were readily elicited fifteen minutes after the decerebration (45 minutes after in the adult). Sections through the caudal third of the thalamus dorsally and through the mammillary bodies ventrally eliminated the alternate rhythmical movements in the forelegs. There was no rigidity after these sections were made and the shock was very slight as the simpler reflexes and the alternate rhythmical movements in the hind legs were present almost immediately after the sections were made. This level of section almost exactly corresponds to the level at which the alternate rhythmical movements in the adult cat were eliminated. When the section passed through the brain stem just cephalad to the superior colliculi dorsally and at the point of exit of the oculomotor nerves ventrally there was no rigidity and the shock produced was very slight if any. Ipsilateral flexion and crossed extension reflexes were present in the fore- and hind legs almost immediately after the section was made. There was no indication of the coördinated movements of progression at any time after the section had been made at this level which is practically identical with that at which the alternate movements in the hind legs of the adult were eliminated.

It is evident that the nervous mechanism which functions in making possible the coördinated movements of progression is present in young animals. Shock and rigidity in these animals are very slight in contrast to the shock and rigidity obtained after similar operations in the adults of the same species. It is apparent then that as the levels at which the coordinated movements of progression are eliminated in the kitten, are practically identical with those at which the movements are eliminated in the adult, it is reasonable to conclude that shock and extreme rigidity are not the factors which eliminate the movements in the adult animal. The elimination of the movements must be due to the removal of some nervous structures in the brain stem whose presence is necessary in order that the nervous mechanism which makes possible the coördinated movements of progression can function normally.

Dogs. *Methods.* The methods were identical with those used for the cat.

Observations. The shock resulting from decerebration through the cephalic third of the thalamus, figure 11, was more profound than that observed in the cat, the decerebration having been done at approximately the same level. The rigidity was quite prominent at first but gradually became less, the hind legs showing a marked clonus following the sections at various levels through the thalamus.

The flexion reflex returned in the forelegs shortly after it was observed in the hind legs. About an hour after the decerebration the animals made

efforts to rise but were unsuccessful. At this time stimulation of the dorsal cutaneous nerves in the thoracic region resulted in violent coordinated movements of progression. The movements were similar to those described for the cat and when the stimulus was applied over an extended period of time (30 to 60 seconds) synchronous bilateral movements were observed in the hind legs which faded into the alternate rhythmical type. The alternate rhythmical movements in the fore- and hind legs persisted for varying periods of time after the withdrawal of the stimulus. The most caudal level at which the section could be made through the thalamus, and still leave intact the mechanism responsible for the coördinated movements of progression, was through the cephalic third of

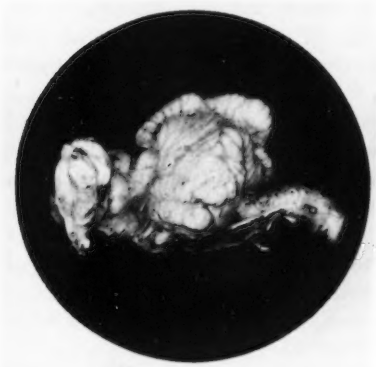

Fig. 11

Fig. 12

Fig. 11. Dog brain, showing the most caudal level through the thalamus at which sections could be made and still obtain coördinated movements of progression.

Fig. 12. Dog brain, showing level of section through the brain-stem at which the coördinated movements of progression were eliminated.

the thalamus dorsally and the cephalic portion of the tuber cinereum ventrally (fig. 11).

When the section passed through the brain-stem just cephalad to the superior colliculi dorsally, and the point of exit of the oculomotor nerve ventrally, figure 12, the rigidity was very marked and there was a marked clonus in the hind legs. The shock was just as profound as when the section passed through the thalamus at the higher levels. After about an hour there was some spontaneous movement of the head but there were no efforts to rise. Stimuli applied to the dorsal cutaneous nerves, pads of the feet and perineum failed to elicit the coördinated movements of progression. Very strong faradic currents applied to the dorsal cutaneous

nerves in the thoracic region failed to elicit the movements but an ipsilateral scratch reflex was produced. At no time during the experiments, which lasted for several hours, was there any indication of the coördinated movements of progression and it was considered that this section had so interfered with the nervous mechanism which makes possible the coordinated movements of progression that it could no longer function.

Pups. *Methods.* Pups, eight weeks old, that were just able to walk were used. The methods employed were identical with those used for the adult dog.

Observations. Shock resulting from the decerebration was very slight as shown by the time taken for the reflexes to return in the legs. There was no rigidity in the hind legs but the rigidity in the forelegs was quite marked and showed a definite increase after the animal had been stimulated and the movements of progression produced. When the animal was allowed to lie quietly on its side the rigidity in the forelegs entirely disappeared but always returned after stimulation.

The sections through the brain-stem which eliminated the coördinated movements of progression in the pups were practically identical with those which eliminated the movements in the adult dog. It is apparent from these results that, as in the cat, shock and rigidity are not the primary factors concerned in the elimination of the coördinated movements of progression. The elimination of the movements is then due to a removal of a portion of the brain stem in the region of the thalamus which interrupts the normal reflex pathways concerned in making possible the coordinated movements of progression.

Discussion. It would be quite superfluous to discuss the phenomenon of shock to any great extent in the present work. The causal observations on shock, which have been noted, will, however, require a brief consideration.

The gradual loss, from amphibians to man, of spinal cord autonomy and the increase of the fore-brain systems over the spinal systems is well known. The observation on shock in the present work indicates that the removal of the cerebral hemispheres and thalamus is intimately related to the phenomenon of shock in cats and dogs and to a less extent in rabbits. The profundity of the effect on the lower systems in the cord is, however, not so marked as when the transection is made through the cord, as Sherrington (15) has already pointed out.

It is a well-known fact that the brain of the carnivor is much more highly developed than that of the rabbit, the process of cephalization having resulted in a greater dominance of fore-brain systems over the spinal systems. It therefore seems plausible that the removal of the cortical and thalamic systems in the cat and dog would result in a more profound effect on the lower stem and spinal systems than in the rabbit where the process of cephalization has not advanced so far.

It may be asked: Can shock and rigidity be ruled out as primary factors in the elimination of the coördinated movements of progression? Babak (1) showed that transection of the cord of tadpoles produced no spinal shock. Pike (10) stated that spinal shock was much less severe in young animals than in older members of the same species and suggested an explanation by assuming that the law of biogenesis applied to function as well as structure and to quote his own words, "Those parts of the central nervous system which are last to appear phylogenetically are last to reach their full functional development."

The observations on kittens and pups in the present work prove that shock and rigidity are much less marked than in the adults following decerebration at corresponding levels. Furthermore the levels of transection through the brain stem at which the coördinated movements of progression were eliminated in the young were practically identical with those at which the coördinated movements of progression were eliminated in the adult. From these experiments on the young and adults of the same species it seems reasonable to conclude that shock and rigidity are not the primary factors concerned in the elimination of the coördinated movements of progression in the animals studied. The elimination of the movements is due to the removal of some nerve structures which function in making possible the coördinated movements of progression. These systems are so arranged that it is necessary that the caudal two-thirds of the thalamus of the cat and dog, and the cephalic two-thirds of the pontine region of the rabbit should be intact to insure the coördinated movements of progression.

The comparatively simple spinal reflexes, noted in ordinary flexion and extension are not nearly so complex as the reflex activities concerned in the coördinated movements of progression. Sherrington (15), using simple flexion and extension as indicators, pointed out that the removal of the cerebral hemispheres did not result in such profound shock to the lower spinal systems as when the level of transection was made caudad to the pons. He considered that normally there was aborally directed influence from the pons and mid-brain nuclei, influenced by visual, equilibratory, and spinal impulses, the interruption of which left the cord systems in the depressed condition of shock as indicated by the absence of the cord reflexes, caudad to the transection, over varying periods of time.

This work shows that section of the cerebro-spinal axis at higher levels produces shock and also eliminates the coördinated movements of progression temporarily. The animal then recovers (cats and dogs more slowly than rabbits, showing the phylogenetic grading of shock), and the simple reflexes return as well as the coördinated movements of progression.

If, following the primary section, another section is made say through the brain stem of the cat, level 2, figure 7, shock may not be so severe although in some animals it was just as profound as that observed after

the primary section. The animal recovered from "shock" in the usual sense of the word as shown by the return of simple reflexes, but in no way could the coördinated movements of progression be elicited. These facts then show, that although shock may account for the disappearance of the simple reflexes and the coördinated movements of progression temporarily, it does not explain the total inability of animals, after sections at various levels, to perform coördinated movements of progression. It was further observed that medial longitudinal sections through the thalamus of the cat, and the mid-brain and pons of the rabbit, resulted in deep shock. The movements of progression were eliminated but the animal recovered from shock in the usual sense of the term. It must be concluded then that some other factor is involved.

There are two alternatives. First, our experiments may furnish an analysis of shock, showing that by using a more elaborate test for shock than that furnished by the simple reflexes, shock of a different nature than that described before may be demonstrated,—assuming as did Sherrington (15), that shock consists of the phenomenon which follows removal of aborally directed impulses from higher to lower centers. Secondly, our experiments may demonstrate that, for the accomplishment of complex coördinated reflexes like movements of progression, there is necessary not only the simpler segmental reflex mechanisms in the spinal cord, but also higher reflex mechanisms whose portals of entry are in the thalamus, mid-brain and medulla.

In order to justify the first possible explanation we must assume a difference in quality of the aborally directed governing impulses. This seems unwarranted in the light of our present knowledge of nervous function. The second explanation seems the more sound and is justified not only by the experimental results but also by an appreciation of the reflex feeding and running reactions of the animals in question.

Shock, resulting from decerebration, has a more profound effect on the cervical and thoracic spinal mechanism than on the lumbar and sacral as shown by the earlier return of reflexes in the hind legs. I have been unable to find any satisfactory explanation for these observations. In the present work it was observed that the coördinated movements in the forelegs were eliminated by the removal of a portion of the brain stem cephalad to the portion whose removal eliminated the movements in the hind legs. Might not this observation be correlated in some way with the observations mentioned above in respect to shock? Further work is in progress on this point.

The observations on spinal rabbits have been fully described above and confirm those of Freusberg (5) and Phillipson (9) for the late spinal dog. These results show that in the late spinal rabbit the lumbar and sacral regions of the spinal cord possess a nervous mechanism which is able to

produce the alternate rhythmical movements in the hind legs but which is unable to produce the bilateral synchronous movements of running and hopping.

Coördinated movements of progression were not obtained in decapitate rabbits; also reflex stepping and synchronous bilateral movements were not obtained in the hind legs of those preparations. Reflex stepping was obtained in the late spinal rabbits as indicated above. The absence of reflex stepping in the decapitate rabbit is peculiar. Sherrington (12) and Miller (8) showed that reflex stepping was prominent in decapitate cats and, as cephalization has not advanced as far in the rabbit as in the cat, one would expect that reflex stepping in the rabbit would be marked. Synchronous bilateral movements in the hind legs of the rabbit are the normal movements in the act of progression. It is probable that these movements have developed in response to the mode of living of the animal and depend for their execution on the dominance of some higher "centers," in the brain-stem, over lower "centers" in the lumbar and sacral regions of the cord. Decapitation and spinal transection eliminate this higher influence which probably leaves the lower centers in a temporary dilemma from which they recover, after several weeks, when they act in their primitive manner by producing the alternate rhythmical movements in the hind legs which resembles the step.

In animals possessing the cerebral cortex it is frequently impossible to foretell, with any degree of certainty, what reaction may be elicited from any definite stimulus. The intact animal is an individual whose actions are guided by a degree of intelligence and influenced by sensations of hunger, fear, pain and the like. Its behavior is analogous to what in man is associated with volition and consciousness. Volitional manifestations have long been associated with the cerebral hemispheres and there seems to exist an exact parallelism between the degree of intelligence of the animal and the development of the cerebral hemispheres.

It is possible to obtain numerous motor integrated movements such as stepping and scratch reflexes from spinal animals. The invariability of response in these automatisms to various stimuli is characteristic. Goltz (6) showed that the dog possessed complete power of locomotion after the cerebral hemispheres had been ablated. The present work shows that, in the cat and rabbit, the power of locomotion is complete after ablation of the cerebral hemispheres and the cephalic portion of the thalamus. These animals appear as mere automatons and respond to nociceptive stimuli chiefly by progression. The movements of progression last for a varying period and their automaticity was remarkably demonstrated in the cat when the animal was standing with its head against the wall while the legs performed coördinated walking movements. These movements must be the result of the spinal cord mechanism being played upon by the

impulses from the cerebellum, thalamus, mid-brain and medulla. The movements while they were more complex than those obtained from the spinal animals, are, nevertheless, more or less fixed or organized. They do not show the phasic changes which exist when the whole cerebrospinal mechanism is intact. Movements of progression in the normal animals are guided and controlled by the cerebral mechanism but it is clear that the cerebral hemispheres are not of primary importance in the performance of the coördinated movements of progression.

The act of progression is highly coördinated, the complex movements depending for their execution on the integrated action of the many nerve centers located at different levels in the cerebrospinal axis. Progression is the result of a sequence of events performed in part consciously and in part unconsciously, any interruption of this normal march of events results in changes in the coördinated movements and, as shown in this work, if the interruption is sufficiently great the movements are eliminated. The harmonious action of the nervous system depends on the integrated action of all its part. During its normal functioning some impulses are inhibited while the passage of others is facilitated and they dominate the reflex pathways.

Graham Brown (2) suggested that the rhythmical phenomenon observed in the reflex step was conditioned centrally and not initiated by any exteroceptive or proprioceptive influence. He considered the rhythm to be the result of two equal and opposite activities in the local nerve centers, one which tends to excite flexion and one which tends to excite extension. Sherrington's work (11) on reflexes and his explanation of decerebrate rigidity gave the first experimental evidence for the law of release of function as presented by Jackson; all the lower centers in the cord being directly under the control of the higher centers in the brain. The work of Freusberg (5), Phillipson (9) on the dog, that of Brown (2), (3), Miller (8) and Sherrington (12), (13) on the cat and the present work on the rabbit, demonstrates that the local centers in the lumbar and sacral regions of the cord are capable of initiating the reflex step but fail to prove that the cord centers are able to produce the coördinated movements of progression. These coördinated movements of progression must be due to the activity of a complex reflex system extending through the spinal cord into the brain-stem and which depends for its normal function on the passage through it of afferent impulses from the cerebral hemispheres and the great sensory receptors throughout the body. The afferent impulses are probably transformed by this mechanism into an aborally directed stream to the lower centers in the cord.

The present work shows that, in the rabbit, the nervous mechanism, which functions in making possible the coördinated movements of progression, is so arranged that the removal of the cephalic two-thirds of the

pontine region eliminates the movements of progression. The corresponding mechanism, in the cat and dog, is so arranged that the removal of the caudal two-thirds of the thalamus and probably a very small part of the cephalic end of the mid-brain eliminates the coördinated movements. The normal functioning of this mechanism depends on the march of events through it, which is initiated by impulses arriving through the great afferent inlets in the cerebrospinal axis. The result is probably an aborally directed stream of impulses which act on the lower cord centers stimulating them in such a manner that there is a definite coördination between fore- and hind legs and if Brown's hypothesis is correct these impulses might act on the cord centers setting up a balanced process of inhibition and excitation which results in a rhythmical discharge to the legs. The uninterrupted activity of this mechanism results in the coördinated movements of progression.

The present work does not prove that there is any definite motor discharging center in the thalamus of the cat and dog, and in the pontine region of the rabbit, which functions in an emanation of a series of impulses which would result in the coördinated movements of progression. It is more probable that these systems are complex systems of reflex pathways, as indicated above, which depend on the interrelation of neurons on either side of the mid-line and so arranged that injury to or ablation of the regions indicated above impairs or eliminates the normal function. Certain types of stimuli give rise to impulses which are able to force the synapses in these systems and which are converted into an aborally directed stream of impulses which plays upon the lower systems in the cord in such a manner that the coördinated movements of progression are produced.

The difference in cephalic extent of the mechanism necessary for the coordination of the movements of progression, in the carnivors and rabbits, may seem difficult to explain. Kappers (7) in his theory of "Neurobiotaxis" assumed that the effector centers are so influenced by the afferent conductors, in the phylogenetic development of the various animal groups, that they migrate toward the locus of the maximal amount of stimulation. It seems plausible that this difference in the arrangement of the coördinating mechanisms for progression in the carnivors and rabbits might be explained by this theory; not that there has been a migration of definite nuclei and fiber tracts, but that there has been a change in the position of the physiological dominance of sensory receiving mechanisms.

The responses of the cat, dog and rabbit to their environment are quite different. The rabbit uses the legs chiefly for locomotion although the hind legs are sometimes used for defense. In obtaining food, no highly complex movements of exactness and precision are necessary because of its herbivorous diet. Its methods of defense against members of its own species require no highly coördinated movements as observed in the

carnivors, and it depends on flight for protection against animals of other species. The rabbit is warned of approaching danger, chiefly through auditory sensations. The preservation of the race depends on the animals' ability to seek safety by flight, hence in accordance with the theory of Neurobiotaxis, we can assume that, during the phylogenetic development of the species, physiological dominance has so changed its position that the arrangement of the higher portion of the nervous mechanism, which makes possible the coördinated movements of progression, is such that it is in close relation to the auditory systems in the pontine region.

In the cat and dog, the movements of defense, stalking, springing and seizing their prey are very exact and precise. The exactness and precision of these movements depend for their execution on visual and probably to a less extent on olfactory and auditory sensations. It is therefore important that there should be a very close relationship between the visual nerve mechanism and the nerve mechanism which is concerned in the coördinated movements of progression. We can therefore assume, in accordance with Kappers' theory, that during the phylogenetic development of the carnivors the physiological dominance has so changed its position that the higher part of the nervous mechanism which makes possible the coördinated movements of progression, is so arranged in the region of the thalamus that a close relationship exists between it and the visual and olfactory systems and to a less extent between it and the auditory systems.

The difference in the arrangement of the dominant sensory systems of the coördinating mechanism for progression might also be explained by the higher cephalization that has taken place in the carnivors. Coghill (4), from his work on Diemyctulus torosus, suggested that, phylogenetically, the most primitive cephalization was in response to the demands of locomotion. This resulted in a "controlling center" in the region corresponding to the myelencephalon or upper part of the medulla. The brain of the rabbit is very primitive and the present work shows that the coördinating mechanism for progression is so arranged that it is intimately related to this primitive region indicated by Coghill. In the cat and dog the corresponding region is in the thalamus. The brain of the carnivor is much more highly developed than that of the rabbit and a higher cephalization has occurred. This higher cephalization has resulted in the cortical and thalamic systems becoming more important as centers of general motor control at the expense of the lower stem and spinal centers. The results of this work are in accordance with this view.

SUMMARY

1. The nervous mechanism which functions in making possible the coordinated movements of progression is so arranged, in the cat and dog, that the caudal two-thirds of the thalamus has to be intact for it to func-

tion normally. The corresponding mechanism in the rabbit is so arranged that the cephalic two-thirds of the pontine region must be intact in order for it to function normally.

2. Definite anatomical centers from which this control might originate were not identified. The controlling influence is probably the result of a march of events through complex reflex pathways so arranged that the portions of the brain-stem indicated in section 1, have to be intact in order that the mechanism can perform its normal function.

3. The necessity for the integrated activity of the neurons on either side of the mid-line is shown by the elimination of the coördinated movements of progression by medial longitudinal section passing through these portions of the brain-stem.

4. The cerebellum is not essential for the production of the coördinated movements of progression in the rabbit. The movements were obtained after complete removal of the cerebellum but they were slightly different in that the forelegs were held in a semiflexed position while they performed the alternate rhythmical movements.

5. Marked rigidity in the rabbit, cat and dog did not inhibit the coördinated movements of progression. The movements appeared to break through and become superimposed on the rigidity.

6. Coördinated movements of progression were not obtained in decapitate animals.

7. The lumbar and sacral regions of the cord, in the rabbit, contain a nervous mechanism which is sufficient to give the alternate rhythmical movements which constitute the reflex step in the hind legs, but is not able to produce the synchronous bilateral movements as observed in the normal act of progression.

8. The alternate rhythmical movements in the forelegs in the cats and rabbits were eliminated by sections through the brain-stem cephalad to the level of the sections which eliminated the movements in the hind legs.

9. Shock resulting from decerebration through the thalamus at the same levels is more profound in dogs than in cats and more profound in cats than in rabbits thus showing the phylogenetic grading of shock.

10. Shock resulting from succeeding section through the thalamus of cats and dogs caudad to the primary sections was just as profound as that produced by the primary section, in the majority of cases. This was not the case in the rabbit where succeeding sections, caudad to the primary sections, through the thalamus and mid-brain resulted in very little shock until the section passed through the caudal portion of the pontine region when the shock became more marked.

11. Kittens and pups showed very little shock and rigidity after decerebration at levels which corresponded with the levels of decerebration in the

adults. The coördinated movements of progression were eliminated in kittens and pups at the same levels as in the adults. It therefore seems plausible that shock and rigidity are not the primary factors concerned in the elimination of the movements of progression.

In conclusion I wish to express my sincere thanks to Dr. F. R. Miller, in whose laboratory the work was done, and to Drs. P. S. McKibben and A. J. Carlson for many helpful suggestions and encouragement.

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THE EFFECT OF CHANGES IN PULSE RATE ON DIASTOLIC HEART SIZE

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A study of the influence that pulse rate has on heart size is of interest practically in the interpretation of clinical x-rays and theoretically for its bearing on certain problems of cardiac dynamics. The work reported here consists of several series of experiments on dogs both anesthetized and unanesthetized in which the diastolic size of the heart under various conditions was followed by means of x-ray silhouettes.

Cardiac output depends upon venous return, heart rate, the contractile condition of the heart musculature and arterial resistance. Cardiac output per beat is determined by the relative diastolic and systolic sizes, that is, by the extent of ventricular filling and by the completeness of ventricular systole. It is obvious that the output per beat need not increase with a greater diastolic size although in most cases it probably does, at least within certain limits so long as the heart is not injured. Our attention in the present paper has been directed solely to the diastolic size. Of the factors concerned in cardiac output in experiments such as ours, three exert their influence by determining the extent of diastolic filling, namely, venous return, the heart rate, and the contractile condition of the heart musculature. In the final analysis it is the effective venous pressure which is of most importance, the other factors only determining the extent to which a given venous pressure is able to fill the heart. Peripheral resistance played no important rôle in our experiments since arterial pressure fell with decreasing heart rates and could not therefore cause dilatation. These problems have been touched upon in a previous publication (1). For a full discussion of all the dynamic factors involved, particularly the controversial questions concerning the shape of the diastolic volume curve and whether a venous pressure above normal results in an increased output per beat, the writings of Wiggers (2) and Henderson (3) should be consulted.

Methods. Dogs were used in all experiments. For the x-ray examinations they were tied back down to an operating board. The x-ray tube was at a distance of one meter. Corrections were made for distortion of the shadow. The position of the animal was carefully maintained

during the course of the entire experiment. The pictures were made by one or more flashes taken during inspiration, the length of exposure, always being long enough to include the diastolic portion of a heart cycle.

The dog is a particularly satisfactory animal on which to make x-ray studies. The heart is placed well above the diaphragm so that in a majority of cases the entire outline of the heart can be followed as a clearly defined line with the exception of the base. If a constant arc is drawn across the base from the angle formed by the great vessels with the right auricle, the heart area on the film can be marked off with great accuracy. Successive observations may be made on a normal dog well within a 5 per cent limit of variation. We have usually not accepted an increase or decrease in the area of silhouettes as being significant unless it reached this figure.

The heart shadow is measured in square centimeters by a planimeter. The error in this determination is very slight indeed, it being possible to measure a heart shadow of 40 sq. cm. repeatedly with a variation of only 0.3 to 0.4 sq. cm. That the silhouette area bears a relation to the volume of the heart can scarcely be doubted and we have therefore used the figures for area to indicate increased or decreased diastolic size. These figures may be translated into actual cardiac volumes by the formula worked out by Skavlem (4).

Variations in heart rate were secured in morphinized animals by injecting atropin and following the return of the rapid pulse to normal. In these cases it was necessary to determine heart rate by use of the stethoscope. Slow rates could often not be secured in this way and there was the further disadvantage that one could not be absolutely sure of the rate at the exact instant the x-ray was taken. In animals under ether anesthesia changes in rate were secured by vagal stimulation after vagotomy. There is probably no doubt that this also produces changes in cardiac contractility, but this is not so objectionable as may at first appear since we believe that normally most changes in heart rate are brought about by increase or decrease of vagal tonus. Veronal was used in a few experiments. In these experiments blood pressure was recorded by a mercury manometer from which the heart rate was accurately determined. The exact instant at which the x-ray exposure was made was also recorded with an electric signal. Venous pressure when taken was measured from a sound inserted into the right jugular and extending into the thorax. The sound was filled with citrate and balanced against a manometer filled with the same solution.

Changes in diastolic size with decreasing heart rates. In all 30 experiments with 266 x-ray observations have been made. The several ways of securing variations in heart rate have all yielded similar results, the gradual recovery of vagal tonus after atropin being quite comparable

to actual stimulation of the vagus by an electrical current. We are quite aware that the anesthetics, ether in particular, have an effect on heart size but this influence was very carefully kept as constant as possible throughout each experiment, and it may be ignored for relative results. The restlessness of the animals often made it impossible to secure satisfactory results without some kind of an anesthetic, even with as simple a procedure as injection of atropin, the important point being that if a series of x-rays is of value the animal must be in exactly the same position for each exposure.

In figures 1 and 2 may be seen the data of two experiments in which the silhouette areas have been plotted against the rates. Minor variations of course occur, but these two curves are typical of the entire series in illustrating two characteristics common to all. Although the x-ray pictures were secured by reducing a rapid heart rate by vagal stimulation or by waiting for the atropin effect to subside it is clearer to discuss the curves as if there was a gradually increasing rate.

It will be noted first that from a rate fairly slow for the dog, 54 per minute in experiment H 62 and 56 per minute in experiment H 45, the heart size for a time decreases but slightly as the rate increases. In the second place there comes a period in which there is a reduction in cardiac area more marked than the increase in rate. For example, in experiment H 62 the area increases only 1 sq. cm. as the rate advances from 54 to 108 per minute. This is within the limits of our accuracy for measurement but since the four x-rays taken indicate this slight decrease it is probably correct. During the next 20 beats the area decreases, however, nearly 3 sq. cm., a very definite and significant change. Twenty-six of our experiments show these two periods and probably all would if the x-ray exposures could have been made at the proper moment. In 22 of the experiments this sudden decrease does not occur until an average rate of at least 104 is reached. In 26 of the experiments it has taken place before the average rate reaches 118. We may say then that in dogs under our experimental conditions at about a rate of 110 per minute a marked reduction in size begins to appear. Up to that time a change in rate has little significance on diastolic size but thereafter a slight increase in rate may be accompanied by a much smaller silhouette area. The chief variation in our curves is the steepness of descent after the heart area has once begun to decrease.

In figure 3 are reproduced x-ray pictures from another experiment illustrating the same points. From a rate of 72 per minute to 108 there is a slight decrease in area from 43.4 sq. cm. to 41.4 sq. cm. During the next 15 beats the area decreases rapidly to 36.9 sq. cm. At a rate of 171 the area is reduced to 34.5 sq. cm. In some experiments 20 or 30 beats after the break the curve tends to taper off into a plateau so that

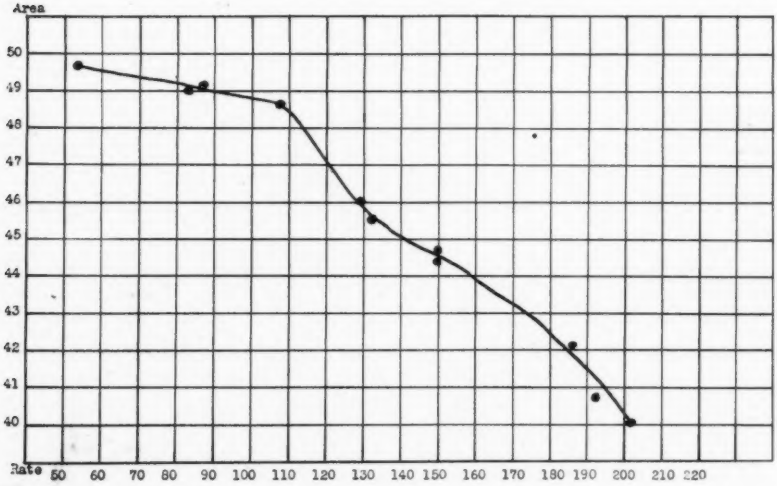

Fig. 1. Data from experiment H 62. Morphine-ether anesthesia. Rates varied after double vagotomy by stimulation of right vagus. Ordinates = corrected silhouette area in square centimeters. Abscissae = rate in beats per minute. Rates determined by blood pressure tracing.

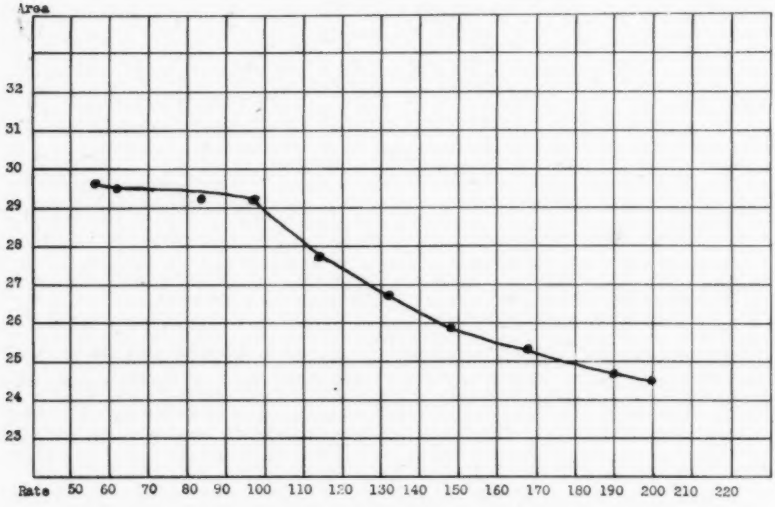

Fig. 2. Data from experiment H 45. Morphine used to secure slow rates. Atropin then given and x-rays made as the effect wore off. Rates determined by stethoscope. Ordinates and abscissae as in figure 1.

again there may be rather large changes in rate without much variation in the silhouette areas. The most likely interpretation of such a result is that the venous return has in some way been maintained in spite of the

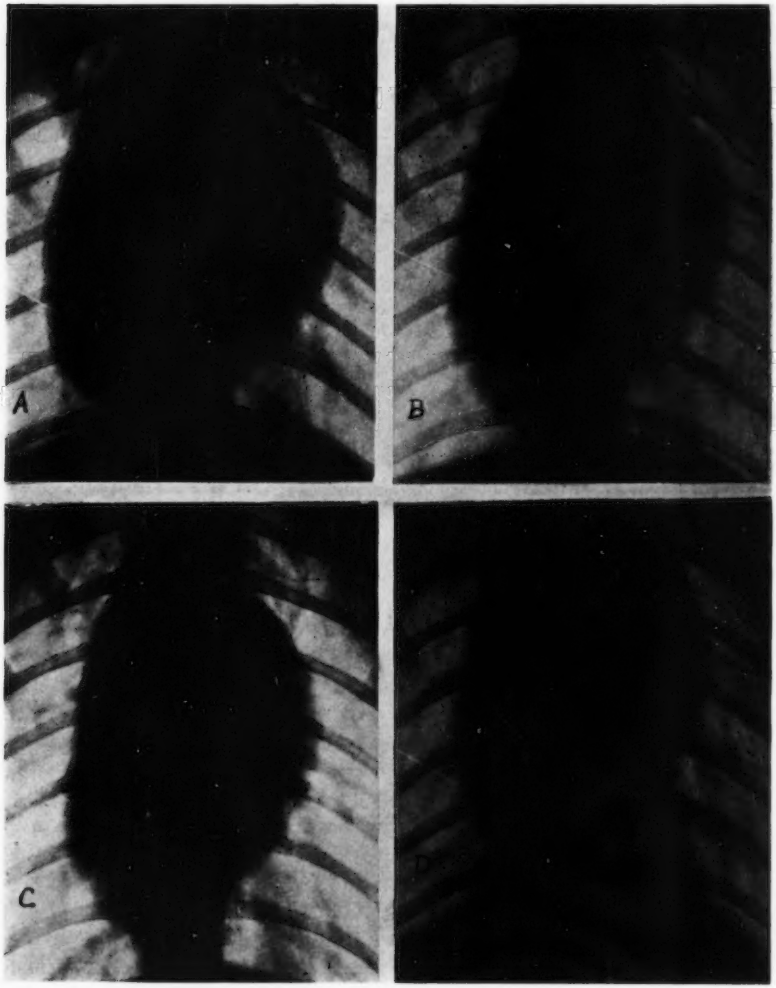

Fig. 3. X-ray silhouettes from experiment H 60. Ether anesthesia. Rates varied by stimulation of right vagus. *A*, 72 beats per minute. Area = 43.4 sq. cm. *B*, 108 beats and area of 41.4 sq. cm. *C*, rate of 123 and area of 36.9 sq. cm. *D*, rate of 171 and area of 34.5 sq. cm.

increasing rate. After a rate of about 180 is reached there is usually little further change in the silhouette areas, as may be seen in experiment H 45. In experiment H 62 this is not quite true although the decrease in size from 192 to 202 beats is not very great.

Variations in heart rate with a constant venous pressure. There may be several factors that aid in causing a reduction of the diastolic size of the heart as its rate increases, but the most important one seems to be the lowering of venous pressure. In the intact animal venous pressure might by certain mechanisms be maintained or made more effective as the pulse rate ascends, but in our experiments circulatory factors are fairly well stabilized, and the venous pressure regularly falls as the rate rises. To find if this is the important factor we have run a series of experiments in which by an infusion of saline-acacia into the left jugular the venous

TABLE 1

To show the effect of varying heart rates with a constant venous pressure. Venous pressure measured in millimeters of H_2O. Area in square centimeters

RECORD NUMBER	VENOUS PRESSURE	HEART RATE	SILHOUETTE AREA
1	20	108	31.6
2	20	114	30.1
3	30	114	31.9
4	30	141	32.4
5	20	141	31.5
6	25	210	32.6
7	20	114	31.7
8	20	60	32.2
9	20	96	31.0
10	20	190	31.7
11	20	115	30.7
12	20	192	31.0
13	25	192	31.9

pressure has been held constant for varying heart rates. The higher the rate the more generous has to be the rate of infusion.

In table 1 may be seen the results of such an experiment. The rate varies from 60 to 210 but even including records 3 and 4 in which the venous pressure rose to 30 mm. H_2O the cardiac area has shown no significant variations. These experiments are carried out with considerable difficulty but we have at least four with results as clear as those submitted. They seem to demonstrate clearly that the reduced cardiac area which appears when the rate is increased is due to the decreased venous pressure.

If venous pressure can be kept high enough the heart fills and distends so rapidly that even a rate of 200 does not show any reduction in diastolic size. But under the conditions of the first series of experiments, with the increase in rate there was a parallel fall in venous pressure, until

at a rate of approximately 110 systole began before filling was complete. It was a question not only of encroachment upon that part of the volume curve known as the period of rapid inflow but a change in its shape, due to the decreased venous pressure.

Discussion. Although one must not read too much into x-ray silhouettes of the heart since they do not have the accuracy of pressure and volume curves the data here presented seem to be of importance and significance in two particulars. In the first place all determinations of heart size by means of the x-ray must bear in mind the effect of varying heart rates. The interesting work on the effects of drugs recently reported by Gordon and Wells (5) would be of more value if the pulse rates were noted. There is little doubt that the same remarks apply to roentgenograms of the human heart. Hodges (6) in this laboratory has recently made some observations along this line. In nine individuals the rate was decreased by the administration of atropin. Heart rates were not secured higher than 130 per minute but in four of the experiments the silhouette area decreased anywhere from 6 to 16 per cent. Practically only the latter figure was of real importance but the conclusion seems justified that the rates must be kept near normal if the measurements are to be of value.

In the second place the data have a bearing on the manner in which the diastolic filling of the ventricles takes place. This has been one of the most discussed questions of cardiodynamics. Henderson (7) was the first to recognize the fundamental effects of changes in heart rate on diastolic filling and consequently on systolic discharge. According to his volume curves there is a short period after the opening of the A-V valves in which under normal venous pressure the ventricles are filled. This interval has been termed the phase of rapid inflow. If the cycle is long enough this phase of rapid inflow is followed by a second stage of gradual filling which Henderson called the *diastasis*. The amount of blood entering the ventricles during diastasis is relatively small, and for this reason the diastolic size and systolic ejection both remain practically constant for every beat until the heart rate becomes fast enough to encroach upon the phase of rapid inflow. Henderson and his fellow workers, believing that all volume curves are superimposable when the venous pressure is at least as great as 50 mm. H_2O, enunciated therefore their law of uniformity of cardiac behavior. According to this conception systolic output and diastolic size vary only with the rate.

Other workers, particularly Patterson, Piper and Starling (8) and Straub (9) have opposed the above ideas and have interpreted their volume curves as showing a constant rate of inflow throughout diastole.

This whole field has been subjected to a number of searching investigations by Wiggers (10) and Wiggers and Katz (11). Very briefly it may be said that although these workers question the law of uniformity of

cardiac behavior, believing that neither diastolic filling nor systolic ejection curves are superimposable during increased venous return, they do agree with Henderson's statement of the manner in which under normal conditions diastolic filling takes place.

The data we have just presented strongly support Henderson's view that diastole normally consists first of a period of rapid inflow and second of diastasis. For agreeing with this almost perfectly we have found that as the heart rate increases from 50 to 110 beats per minute there is a slow gradual reduction in diastolic size but as the rate increases above 110 there is a very rapid reduction in the diastolic area. In other words the diastolic size remains fairly constant until the period of rapid inflow is encroached upon. This data seems to be of particular interest and importance because it has been secured without any interference with the heart such as is necessitated by all plethysmographic methods. In all experiments the thorax was intact and in one series there were no operative procedures whatever.

If Henderson's volume curves during vagal stimulation be analyzed (6, page 349) it will be noted that the reduction in diastolic size should begin to be apparent when the rate reaches 100 beats per minute. In our experiments this critical point is close to 110, the difference being probably due to less trauma and hemorrhage in our animals. Both figures however are at a lower rate than would be expected from Wiggers' analyses of the cardiac cycle. There are certain difficulties in deducing rates from the time values of the cardiac phases but if the minimum values given by Wiggers (9) which are the most accurate available are used it will be found that if an auricular systole of 0.077 second is allowed to fall in the period of rapid inflow the heart should reach a rate of slightly over 200 before this period is encroached upon. If the auricular systole is allowed to follow the inflow period of 0.048 second the rate should reach 170 before the filling time of the ventricle is shortened. Now these rates are very considerably in excess of the actual ones at which diastolic filling is reduced as shown by our x-ray pictures and as may be deduced from Henderson's curve. This discrepancy is due to the fact that as the rate increases venous pressure falls, a factor not considered in the above calculations. Wiggers and Katz (10) have shown that the period of rapid inflow shortens with a rising venous pressure. It must then lengthen as pressure falls. This lengthening of the period seems to explain why in practice a rate of about 110 instead of 170 marks the beginning of the decrease in diastolic size. In confirmation of this we have our positive experiments showing that if venous pressure is maintained the heart may reach rates of 200 without decreasing in diastolic size. Not only the height attained by the rapid inflow phase of the volume curve but the time required to reach this height become important in interpreting the effects of changes in heart rate. It is obvious that

our data are explicable only on the assumption that diastolic filling is influenced by variations in venous pressure. The level at which venous pressure changes may become effective is not clear from the experiments presented here, but that it is not probably as low as 50 mm. H_2O may be seen from the work of Wiggers and Katz (10) and observations we have previously made (1).

SUMMARY

In dogs under ether anesthesia with the thorax intact the diastolic size of the heart as shown by the x-ray slowly decreases in size as the heart rate increases from 0 to about 110 beats per minute. As the rate increases beyond an average of 110, the diastolic size undergoes a very rapid decrease.

It is evident from the figures that the heart rate must be carefully considered in any studies made on the heart by the x-ray.

The data presented confirm Henderson's conception that the diastolic filling of the heart under normal conditions consists of two stages, one of rapid inflow and the other of diastasis. This is shown to be true for intact unanesthetized animals.

If venous pressure is maintained artificially the heart rate may be increased as high as 200 beats per minute without any change in diastolic size. This figure agrees with that secured by calculation from the time values of the various phases of the cardiac cycle. Ordinarily the marked decrease in diastolic size appears at a low rate, 110, due to the effects of the falling venous pressure.

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INCREASED BLOOD UREA CONCENTRATION OF EXTRARENAL ORIGIN

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When there is a pronounced loss of water from the body, or when there is a prolonged abstention from fluids, an increased concentration of the substances dissolved in the body fluids might be expected. It is certain, however, that the actual concentrations observed under such conditions are not susceptible of any very simple explanation for while with some substances, e.g., chlorides, the increase in concentration is much less than might be expected, one substance at least—urea—reaches a concentration much greater than can be accounted for by any possible loss of water from the body. This increased concentration of urea in the blood of animals deprived of sufficient water was first noted by Bang (1) in rabbits undergoing complete starvation. High blood urea concentrations are also found in young infants under conditions involving excessive loss of water from the body (2). The experiments described in this paper were undertaken to determine the effect on the blood urea concentration and the rate of urea excretion of abstention from water or of such abstention combined with loss of water induced by diuresis following sucrose injections.

Male rabbits of from 2.3 to 3.2 kilos in weight were used. Albuminuria is of common occurrence in apparently normal animals and all rabbits giving this sign or having an abnormally high blood urea concentration were discarded. No casts were detected in any pre-experiment urines. The rabbits were kept in a well-ventilated, heated room (70° to 75° F.) in individual cages provided with means for collecting voided urine. This was received in a known amount of acid. Most specimens were obtained by catheter and these and the cage collections and the washings of each were all treated separately so that the urine volume and urine urea concentration as well as the rate of urea excretion could be accurately calculated. Blood samples were obtained from the ear vein, usually at the beginning of each urine collection. Weighings were made following catheterization. Urine and blood urea determinations were made by the usual urease methods. The amount of sucrose in some specimens was determined by a special curve applied to a recent method (3).

All rabbits were starved but allowed water for 48 hours before the beginning of the first period of the experiment. The length of time of the

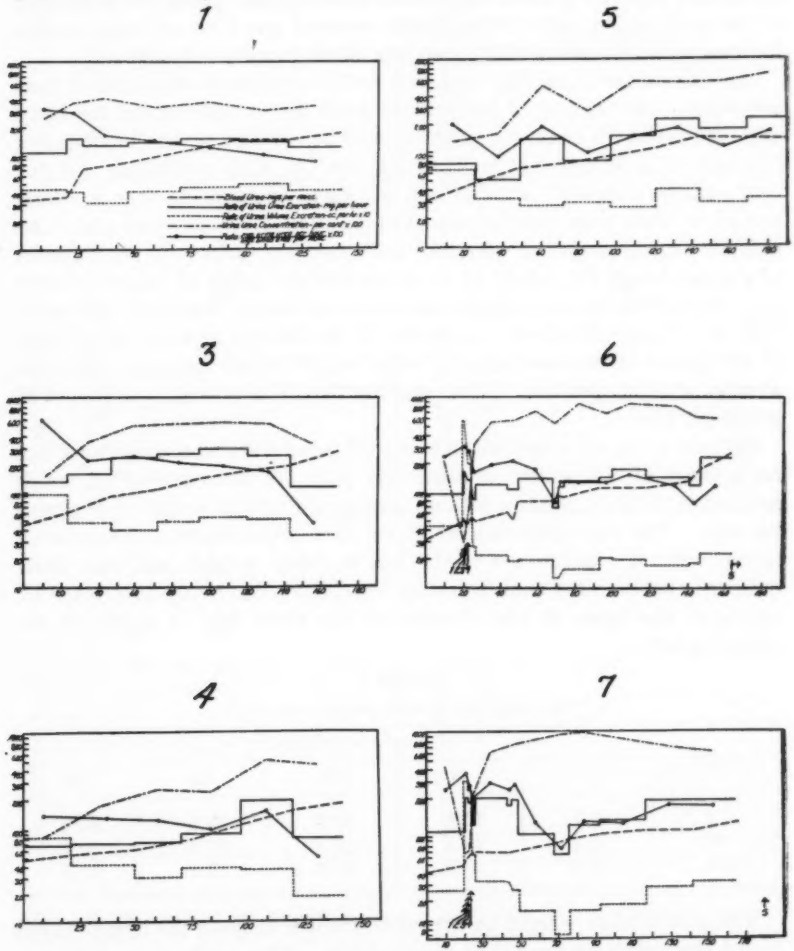

Fig. 1. Logarithmic curves of individual experiments, permitting a comparison of several factors with different units. Time in hours. R.6—1,2, 3 and 4 = 10 cc. 50 per cent sucrose intravenously; 5 = food and water. R.7—1, 2, 3 and 4 = 10 cc. 50 per cent sucrose intravenously; 5 = death.

experimental periods, over which the urine volume and urea excretion were measured, varied. These periods were quite short during diuresis,

while during the greater part of the experiment they were either 12 to 15 or 20 to 30 hours in duration. The periods were lengthened in order to reduce the amount of blood taken from each animal. After the beginning of the first period none of the rabbits received any food or water except for measured amounts of fluid given near death in some instances.

Our first series of animals (exper. 1 to 5) were simply deprived of food and water. In the rest of our rabbits acute loss of water from the body was induced at the commencement of each experiment by the intravenous injection of a 50 per cent sucrose solution (4), (16) containing 0.9 per cent sodium chloride. Rabbits will not stand an injection over any long period of time; even small amounts of material given this way place the animals beyond recovery. By the rapid injection (2 to 4 cc. per minute) of a single (series III, exper. 11 to 16) or divided (series II, exper. 6 to 10) large dose of the sucrose solution an enormous diuresis was easily produced with no obvious ill effects. In series II the rabbits received an average of 8.0 grams of sucrose per kilo body weight which induced a diuresis averaging 54 cc. per kilo. The resulting loss of body weight averaged 56 grams per kilo.

Rabbits 11 to 16, constituting series III, were given in a single injection an average of 7.3 grams of sugar per kilo. A diuresis resulted which amounted to 59 cc. per kilo with an average decrease in weight of 61 grams per kilo. The only indication which we have of the degree of dehydration in our various experiments is the loss in body weight, and that little difference between the three series of experiments can be demonstrated by means of this index of the severity of the water loss is shown in the following table.

TABLE 1

Average loss in body weight—per cent

SERIES	HOUR OF EXPERIMENT				
	50	75	100	125	140
I	12.7	16.6	22.4	26.9	30.9
II	13.1	18.3	22.9	27.2	30.7
III	14.4	18.9	23.5	26.9	29.8

The loss of body weight by the rabbits which suffered the large diuresis is not very different from that of the others. This might be explained in part by the utilization of some of the injected sucrose. In our experiments (table 3) the injected sucrose was not recovered quantitatively in the urine.

The most striking feature of all our experiments was the very marked increase in the urea concentration of the blood. This reached a height of 450 mgm. per 100 cc. of blood in one case (exper. 12) while the animal

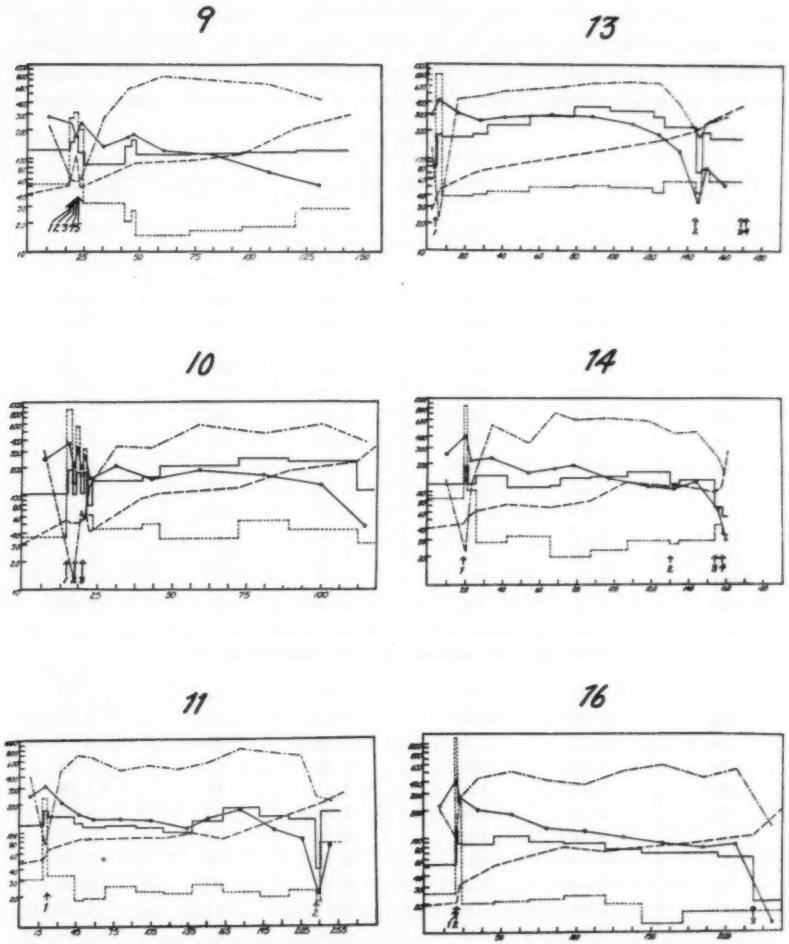

Fig. 2. R.9—1, 2, 3, 4 and 5 = 10 cc. 50 per cent sucrose intravenously. R.10—1 = 20 cc. 50 per cent sucrose intravenously; 2 = 15 cc. same; 3 = 10 cc. same. R.11—1 = 25 cc. same, 2 = 200 cc. physiological salt solution. R.13—1 = 45 cc. 50 per cent sucrose intravenously; 2 = 200 cc. physiological saline by S. T.; 3 = 200 cc. same. R.14—1 = 40 cc. 50 per cent sucrose; 2 = 100 cc. water; 3 = 100 cc. water; 4 = 200 cc. physiological saline. R.16—1 = 40 cc. 50 per cent sucrose; 2 = 15 cc. sucrose, 3 = 200 cc. water.

was still without water. The figures which were observed after giving fluids and which were found to be still higher will be considered later. In table 2 an average of the blood urea and of other observations of each series of experiments has been attempted. The blood urea concentration

TABLE 2*

HOUR OF EXPERIMENT	URINE VOLUME PER HOUR	URINE UREA PER HOUR	URINE UREA CONCENTRATION	BLOOD UREA PER 100 CC.	RATIO: URINE UREA / BLOOD UREA
	Series I. An average of experiments 1 to 5				
	cc.	*mgm.*	*per cent*	*mgm.*	
10	6.87	96	1.52	43	2.27
20	6.86	106	1.74	50	2.26
30	3.85	98	2.60	59	1.54
40	3.85	117	2.60	66	1.54
50	3.38	130	3.62	73	1.58
60	3.38	130	3.62	79	1.58
70	3.38	130	3.62	85	1.58
80	3.76	142	3.74	95	1.37
90	3.76	142	3.74	105	1.37
100	3.87	184	4.86	113	1.52
110	3.87	184	4.86	122	1.52
120	3.99	181	4.93	131	1.48
130	3.61	173	4.77	141	1.33
140	3.61	173	4.77	152	1.33
	Series II. An average of experiments 6 to 10				
10	3.68	114	3.41	44	2.58
20	38.12	167	0.53	60	2.86
25	11.77	114	1.04	49	2.03
30	3.84	106	2.89	60	1.68
40	3.10	135	4.51	65	2.08
50	3.20	135	4.59	73	1.96
60	2.59	162	6.23	82	2.16
70	2.10	140	6.96	89	1.59
80	1.81	115	6.82	97	1.20
90	2.46	155	7.15	111	1.42
100	2.22	154	7.39	127	1.32
110	2.52	158	6.52	138	1.22
120	2.50	165	6.86	149	1.27
130	2.41	141	6.06	183	1.12
140	2.83	155	5.52	231	1.07

*These averages were carried only so far as observations were obtained for every animal in the series. In series I this gives a true average from the first hour. The other averages are more approximate as regards hours, the data from each rabbit being used in the average in such a manner that the ten hour point of the average came in the first period, the twenty hour at the height of the diuresis and the twenty-five hour in the lesser diuresis following.

Serise III. An average of experiments 11 to 16

HOUR OF EXPERIMENT	URINE VOLUME PER HOUR	URINE UREA PER HOUR	URINE UREA CONCENTRATION	BLOOD UREA PER 100 CC.	RATIO: URINE UREA / BLOOD UREA
	cc.	*mgm.*	*per cent*	*mgm.*	
10	4.68	105	2.52	41	2.59
20	77.90	162	0.28	50	3.47
25	11.07	109	1.90	57	2.01
30	2.91	116	3.99	63	1.94
40	3.20	132	4.28	68	2.12
50	2.81	122	4.41	77	1.81
60	2.59	126	5.03	78	1.68
70	2.60	125	4.96	78	1.66
80	2.48	140	5.28	83	1.64
90	2.48	140	5.28	89	1.64
100	2.48	157	5.34	102	1.57
110	2.85	169	5.44	119	1.43
120	2.80	168	5.37	130	1.43
130	2.88	175	5.79	148	1.43
140	2.96	181	5.77	169	1.49
150	2.97	176	6.10	235	1.39

at the 140th hour was 152, 231 and 169 mgm. per 100 cc. of blood for series I, II and III, respectively.

The general trend of our averaged experiments (figs. 3, 4 and 5) reveals the existence of a quantitative relationship between the concentration of urea in the blood and the rate of urea excretion during the first 140 hours. In series II the increase in the rate of urea excretion is irregular but the major change is for a rising rate of urea output. In those experiments of more than 140 hours duration the tendency is for a continuation of this relationship between the blood urea concentration and the rate of urea excretion. The slight decrease in the ratio: $\frac{\text{urea in one hour's urine}}{\text{urea in 100 cc. of blood}}$ during the period of dehydration shows that the relationship is not direct but that on the average the increase in the rate of urea excretion does not quite keep pace with the rising blood urea concentration. The ratio values vary with changes in the conditions, and are highest during the diuresis from sucrose injections, and higher at the commencement of the experiment than when the effect of deprivation of food and water began to be felt. These changes are of a similar nature to those recently reported in man by Addis and Drury (5).

Early in the experiments the urine volume excretion falls rapidly, later slowly, and finally it increases somewhat. The average changes (table 2) are very insignificant giving a urine volume excretion which tends to remain constant. There has been some disagreement in regard to the

TABLE 3

Representative protocols from each series of experiments. The remaining experiments are represented graphically in figures 1 and 2

Series I. Rabbit 2

HOUR OF EXPERIMENT	URINE VOLUME PER HOUR	URINE UREA PER HOUR	URINE UREA CONCENTRATION	BLOOD UREA PER 100 CC.	RATIO: URINE UREA / BLOOD UREA	WEIGHT
	cc.	*mgm.*	*per cent*	*mgm.*		*grams.*
0				37.2		3225
→ 25.0	5.8	98.1	1.66	(41.0)	2.39	
25.0				44.9		3050
→ 48.0	4.1	87.7	2.23	(50.7)	1.73	
48.0				56.6		2850
→ 73.0	3.1	66.5	2.16	(62.0)	1.07	
73.0				67.4		2700
→ 96.0	2.7	127.7	4.68	(78.9)	1.62	
96.0				70.5		2600
→120.0	2.7	134.8	4.98	(78.9)	1.71	
120.0				67.4		2500
→143.0	2.9	150.1	5.18	(72.6)	2.07	
143.0				78.2		2400
→168.0	3.1	169.0	5.43	(82.6)	2.05	
168.0				87.6		2250
→192.0	2.8	182.3	6.50	(93.4)	1.95	
192.0				99.2		2150
→213.0	3.4	236.0	7.04	(107.7)	2.19	
213.0				116.3		2000*
→239.25	4.4	126.0	2.85	(120.9)	1.04	
239.25				125.5		2100*
→261.25	7.3	245.0	3.33	(134.0)	1.83	
				142.5		1950

Series II. Rabbit 8

HOUR OF EXPERIMENT	URINE VOLUME PER HOUR	URINE UREA PER HOUR	URINE UREA CONCENTRATION	BLOOD UREA PER 100 CC.	RATIO: URINE UREA / BLOOD UREA	SUCROSE IN URINE	WEIGHT	
	cc.	*mgm.*	*per cent*	*mgm.*		*per cent*	*grams*	
0				51.4			2,350	
→ 18.5	2.48	134	5.5	(45.9)	2.91	Neg.		
18.5				40.4				10 cc. 50 per cent sucrose
19.5				47.1				10 cc. 50 per cent sucrose
→ 20.5	18.50	144	0.78	↓	3.07	8.90	2,300	10 cc. 50 per cent sucrose
21.5				58.2				
→ 23.0	9.08	125.6	1.37	↓	2.16	8.40	2,250	
→ 24.75	2.43	78.4	3.20	(56.5)	1.39	14.60		
24.75				54.8				
→ 42.75	2.46	123.0	4.99	(57.5)	2.14	19.40		
42.75				60.3			2,200	

*100 cc. H_2O by S. T.

TABLE 3—*Continued*

Series II. Rabbit 8—*Continued*

HOUR OF EXPERIMENT	URINE VOLUME PER HOUR	URINE UREA PER HOUR	URINE UREA CONCENTRATION	BLOOD UREA PER 100 CC.	RATIO: URINE UREA / BLOOD UREA	SUCROSE IN URINE	WEIGHT	
	cc.	*mgm.*	*per cent*	*mgm.*		*per cent*	*grams*	
→ 45.25	2.83	169.0	5.98	(61.8)	2.72	10.30		
→ 48.84	2.28	141.6	6.31	(64.0)	2.21	Neg.		(6.37 grams sucrose per kilo)
48.84				66.2			2,150	
→ 66.5	2.34	139.0	5.84	(75.1)	1.85			
66.5				84.0			2,100	
→ 74.5	2.15	124.2	5.78	(88.6)	1.40			
74.5				93.2			2,075	
→ 97.0	2.52	150.0	5.97	(90.4)	1.66			
97.0				87.7			1,975	
→120.0	2.78	141.0	5.05	(89.0)	1.58			
120.0				90.4			1,900	
→144.0	Lost			(114.4)				
144.0				138.4			1,750	
→168.0	4.03	214.0	5.33	(142.2)	1.50			
168.0				146.0			1,650	
→192.33	4.42	170.0	3.84	(140.7)	1.21			
192.33				135.5			1,550	
→210.33	2.42	96.0	3.94	(246.5)	0.39			
210.33				357.5			1,525	
→218.0	3.66	115.0	3.15	(367.5)	0.31			
218.0				377.5			1,500	

Series III. Rabbit 12

0				39.0			2,800	
→ 20.33	4.24	152.5	3.59	(46.9)	3.25	Neg.		40 cc. 50 per cent sucrose
20.33				54.8				
21.33				72.6				
→ 22.33	95.0	145.5	0.15	↓	2.05	1.20	2,500	
23.33				59.6				
→ 24.58	13.6	61.3	0.45	↓	1.03	1.38	2,450	
25.88				56.8				(7.15 grams sucrose per kilo)
→ 27.83	2.89	20.0	0.69	(53.5)	0.37	10.20		
27.83				45.9				
→ 43.33	4.63	114.0	2.47	(77.7)	1.47	7.50		
43.33				109.5			2,300	
→ 49.50	1.75	88.2	5.04	(106.7)	0.83			
49.50				104.0			2,275	
→ 67.50	1.59	86.1	5.42	(94.5)	0.91			
67.50				85.0			2,200	

TABLE 3—*Concluded*

Series III. Rabbit 12—*Continued*

HOUR OF EXPERIMENT	URINE VOLUME PER HOUR	URINE UREA PER HOUR	URINE UREA CONCENTRATION	BLOOD UREA PER 100 CC.	RATIO: URINE UREA / BLOOD UREA	SUCROSE IN URINE	WEIGHT	
	cc.	*mgm.*	*per cent*	*mgm.*		*per cent*	*grams*	
→91.35	1.03	55.7	4.58	(92.5)	0.62			
91.35				100.0			2,125	
→115.35	1.17	63.9	5.44	(162.5)	0.39			
115.35				225.0			2,025	
→141.35	0.10	6.3	6.30	(329.0)	0.02			
141.35				433.0			1,850	
141.52								Food and water
→169.85	0.34	13.9	4.16	(441.0)	0.03			
169.85				449.0				
215.85				758.0				Died

Series 3. Rabbit 15

0				48.7			2,450	
→ 2.85	3.71	107.6	2.9	50.0	2.15	Neg.	2,450	
2.85				51.4				
3.17				49.3				↓40 cc. 50 per cent sucrose
→ 4.67	69.20	162.0	0.22	(50.0)	3.24	5.70		
4.67				50.7			2,350	
→ 6.67	10.35	63.0	0.61	(56.1)	1.12	12.60		
6.67				61.6			2,350	(8.16 grams sucrose per kilo)
→ 30.0	2.46	117.0	4.75	(63.7)	1.84	Neg.		
30.0				65.8			2,225	
→ 49.08	2.45	102.8	4.18	(68.5)	1.50			
49.08				71.2			2,150	
→ 72.66	2.28	143.5	6.31	(82.1)	1.75			
72.66				93.1			2,100	
→ 97.32	3.77	232.8	6.16	(108.8)	2.13			
97.32				124.6			1,950	
→125.32	3.45	219.5	6.37	(139.8)	1.57			
125.32				155.0			1,850	
→151.20	2.86	221.3	7.84	(196.2)	1.13			
151.20				237.4			1,700	
								Death during night

effect of changes in urine volume on the rate of urea excretion. Ambard and Weill (6) on the basis of a quite insufficient number of observations concluded that when the blood urea concentration was constant the rate varied inversely as the square root of the concentration of the urea in the

urine. Austin, Stillman and Van Slyke (7) found that their results were best explained on the supposition that the rate increases in proportion to the

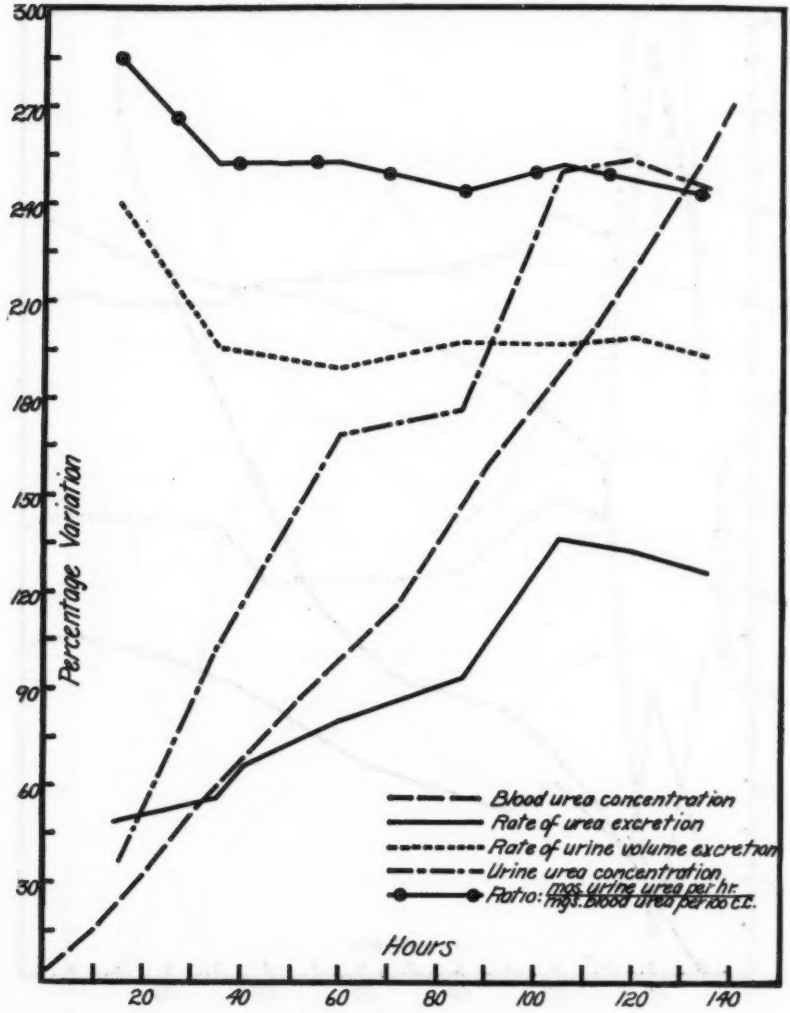

Fig. 3. Based on the average results of series I, experiments in which the animals were simply deprived of food and water. Ordinates denote per cent changes, observations at the tenth hour being selected arbitrarily as 100 per cent.

square root of the urine volume when the volume was less than a certain value, which varies in different individuals. The conditions of our

experiments which produced a transition from high or normal urine volumes to the smallest possible are favorable for testing such hypotheses. The

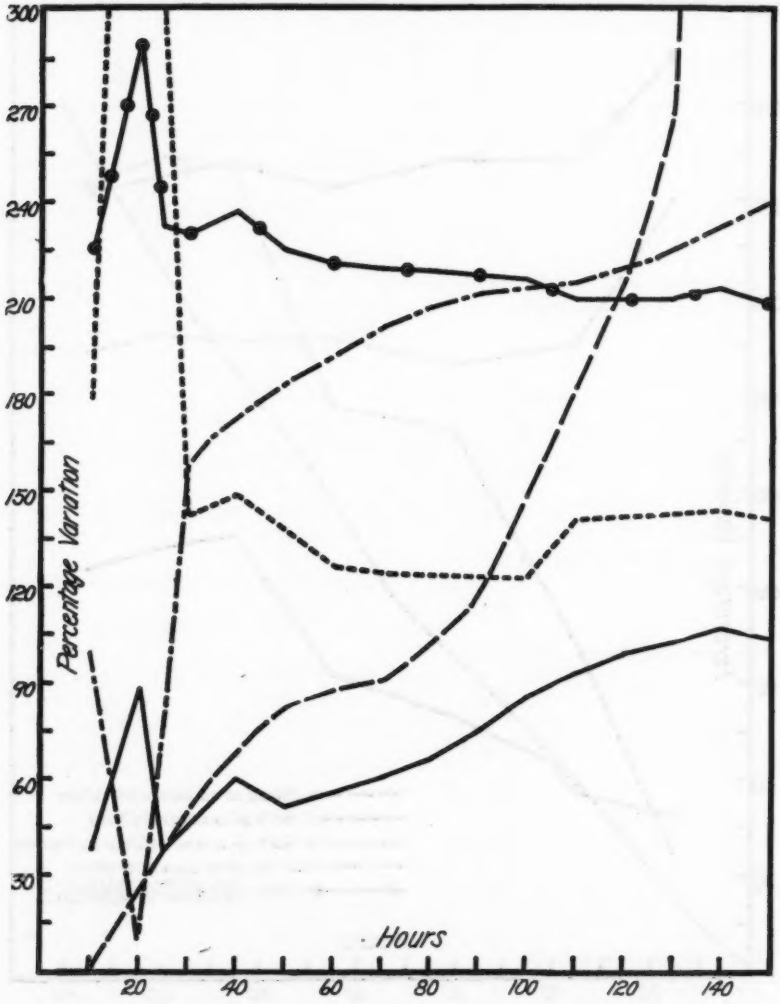

Fig. 4. As in figure 3, the average results of experiments 6 to 10, series II based on the figures of table 2.

results show that neither is confirmed and support the conclusion reached by Addis and Drury (8) that the urine volume as such has no appreciable

effect on the rate although both volume and rate may increase or decrease together with changes in the conditions of the experiment.

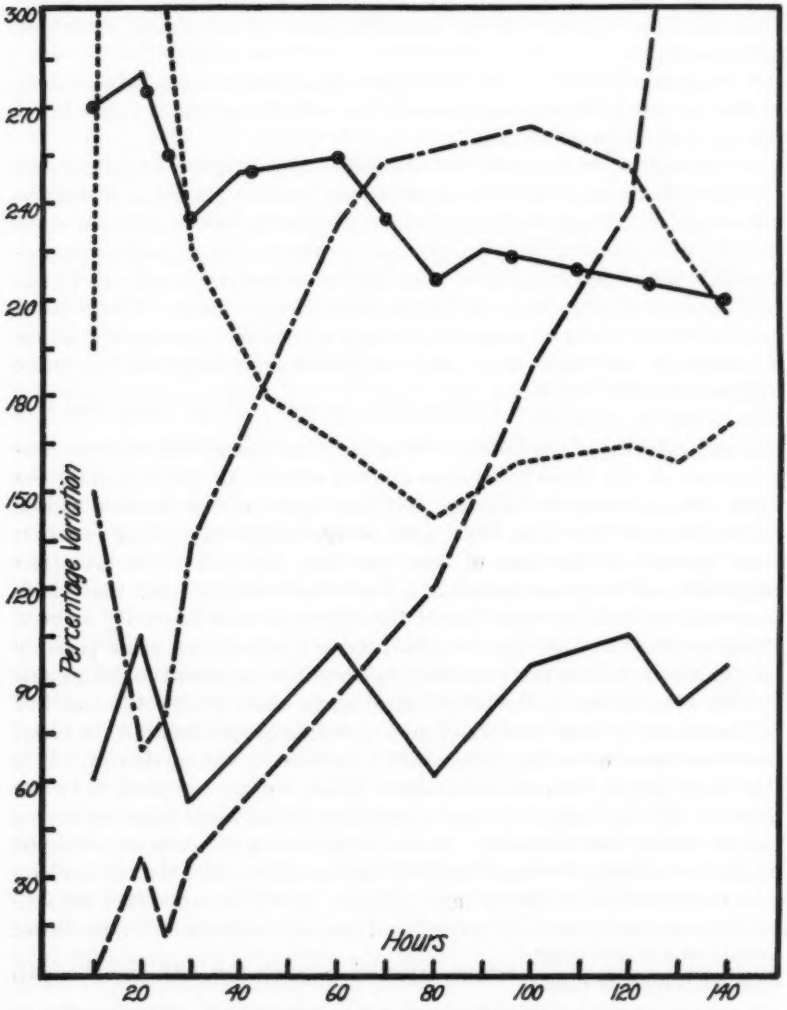

Fig. 5. Averages of series III, experiments 11 to 16, based on table 2. See figure 3

The statement has often been made that the concentration of urea in the urine has a fixed maximum which is never exceeded, and as a corollary it might be assumed that under the conditions of our experiments this

maximum would be reached and maintained. If that had been the case the rate of urea excretion would necessarily have varied directly with the volume of urine. Our results (fig. 1) show however that this maximum concentration is either never attained, or if it is reached, it is not maintained.

Certain conclusions may be drawn from these data in regard to the cause of the increase in blood urea concentration which was such a pronounced feature in all the experiments.

In discussing the reason for the high blood urea concentrations found in infants suffering from water loss produced by gastro-intestinal disorders, certain writers (9), (10), (11), (12) assume the sole or principal cause to be a failure of renal function although no proof of this hypothesis is presented. Now it has been shown that abstention from food and water leads to a decrease in the urea excreting activity of the kidney. Under these conditions the rate of urea excretion at any given blood urea concentration is somewhat less than when food and water are taken, and the ratio: $\frac{\text{urea in one hour's urine}}{\text{urea in 100 cc. of blood}}$ is consequently lowered. It is evident that this lessened activity of the kidney will result in an increase in the urea concentration of the blood if the same amount of urea continues to enter the blood stream from the tissues. But the degree of this increase will be limited because the rising blood urea concentration will in itself cause an equal increase in the rate of urea excretion and the blood urea concentration will become constant at a level which is higher than that which previously existed in proportion to the degree of renal inactivity induced by the conditions. This physiological process of adaptation might possibly be regarded as a form of "renal failure," but it is important to distinguish it from renal failure in the pathological sense which implies the inability of the kidney to keep the rate of urea excretion proportional to the blood urea concentration at the plane which is induced by the conditions. It is the pathological form of renal failure which is often supposed to be the cause of the high blood urea concentrations found when there has been a loss of water from the body. In our experiments the data we collected enable us to determine quantitatively the extent to which the rise in blood urea concentration is due to renal failure. It will be noted that the rate of urea excretion relatively to the blood urea concentration, i.e., the ratio: $\frac{\text{urea in one hour's urine}}{\text{urea in 100 cc. of blood}}$ becomes smaller between the 20th and the 50th hours as the deprivation of food and water takes effect. The rate of urea excretion itself remains constant and the decrease in the ratio is due to the fact that the blood urea concentration rises from about 40 to about 60 mgm. per 100 cc. (table 2). All these changes are in consonance with what might have been expected from observations previously made (5).

They represent a physiological response of the kidney to the conditions induced by abstention from food and water under which the renal activity in the excretion of urea is decreased.

The initial moderate increase in blood urea concentration is thus accounted for but the subsequent continuous increase must have another explanation. If renal failure in the pathological sense is the cause, the rate of urea excretion should fail to maintain the relation to the blood urea concentration which was reached after the deprivation of food and water had taken effect and the rate should decrease, remain constant or at least not increase in proportion to the increase in blood urea concentration in accordance with the degree of renal failure which may exist. Such a process would be marked by a decrease in the magnitude of the ratio: $\frac{\text{urea in one hour's urine}}{\text{urea in 100 cc. of blood}}$ and the extent of the failure would be measured by the steepness of the falling ratio curve. If the average ratios after simple abstention from food and water are consulted (table 2, series I) it will be seen that from the 30th hour onwards there is no fall in the ratio values. The ratio remains almost constant up to the end of the experiment. The high levels of blood urea concentration which were attained were therefore not due to any failure of the kidney to excrete urea.

In series II, the experiments in which repeated sucrose injections were given, there is a decrease of about 50 per cent in the magnitude of the ratio from the 60th to the 140th hour, and in series III in which a single sucrose injection was given there is a 16 per cent decrease. Albumen was not found in the urine of the rabbits which were simply deprived of food and water until the third day when the blood urea concentration was already much above normal, but it appeared immediately after the sucrose injections and in considerably larger amounts as judged by qualitative tests. In the only quantitative determination made the very high concentration of 2.8 per cent was found. Casts were found in only one specimen in the urines after simple dehydration, but large numbers were constantly found after repeated sucrose injections and a considerable though smaller number after the single injections. They were epithelial, granular and hyaline but mainly the latter. We therefore have evidence which suggests that the sucrose injections may have led to structural changes in the kidney although no abnormality was found in the 10 animals whose kidneys were examined microscopically. A study of the individual experiments shows that in some of the sucrose experiments renal failure was a contributory cause of the increased blood urea concentration though in several cases its effect was slight, and in no one of them is a failure of the kidney to excrete urea an adequate explanation of the high levels of blood urea concentration which are attained.

Among extrarenal causes which might have contributed to the decrease in the ratio: $\frac{\text{urea in one hour's urine}}{\text{urea in 100 cc. of blood}}$ seen in some of our experiments the circulatory changes should be mentioned. In all of our animals as the experiment progressed the pulse rate fell markedly often being as low as 25 per minute near death. Other experiments (13) on rabbits have shown that during complete starvation with deprivation of fluids, the blood volume falls below the normal value for the body surface as the result of water loss from the blood. Keith (14), (16) also found a decreased blood volume following water abstinence. An increased concentration of the blood may also have been a factor (15). Unfortunately no accurate method for determining the degree of blood concentration was available. By the use of protein method later found to be inaccurate no increase in serum or whole blood protein was found in either series of rabbits with the exception of a slight increase immediately following the sucrose diuresis. Total solid determinations gave similar results. In a recent paper (16) on the experimental dehydration of dogs by means of intravenously injected sucrose solutions Keith reports marked increases in blood concentration. Utheim (13) found an increased protein and hemoglobin content of the blood in rabbits undergoing complete starvation. However toward the end of all our experiments the blood became notably less viscous and thinner than normal, and the continued bleeding, sometimes reaching 20 cc. or more daily, is an additional factor which must have tended to counteract any tendency toward blood concentration. The fall in the urea ratio immediately following the extreme diuresis (figs. 1 and 2), from which recovery is rapid, might be explained on the basis of the increased viscosity of the blood (16) interfering with renal function (15). But insufficient blood supply to the kidneys either from the circulatory failure or from increased viscosity of the blood could not account for the rise in the blood urea concentration. For if they did the increased concentration of urea in the blood should be due to the inability of the kidney to get the urea to excrete. In that case the ratio would vary inversely to the blood urea concentration which it does not. On the other hand circulatory failure might easily explain the decrease in the ratio that was present in some experiments.

No evidence has ever been brought forward to show that it is the function of the kidneys to maintain a "normal" concentration of urea in the blood, but we know that the rate of urea excretion tends to have a direct relation to the concentration of urea in the blood. When the blood urea is increased temporarily, i.e., by the ingestion of urea, the rate of urea excretion is increased and this naturally leads to a decrease in the height of the urea concentration of the blood if there is no further increase in the amount

of urea entering the blood stream. But a continued administration of urea would lead to a constantly high blood urea concentration.

The high blood urea concentrations which we observed might therefore be accounted for by a marked increase in urea formation. This is the only possible explanation of the increased blood urea concentration for the rate of urea excretion increased throughout every experiment and with this steadily rising urea output the urea intake as far as the body is concerned remains at zero. The urea intake of the blood must increase progressively during our experiments and our curves could no doubt be duplicated as far as blood urea concentration, rate of urea excretion, and ratio are concerned, by continuously administering increasing amounts of urea over the same period of time. The conditions of our experiments have been shown by other workers to lead to an increase in the rate of urea formation. When water hunger is combined with food hunger the effect of the lack of water is more pronounced (17), (18). Deprivation of water alone has an accelerating influence upon the protein catabolism. This was shown by Straub (19), (20) in dogs which were receiving sufficient food but lacked the fluid necessary for normal metabolism. Experiments of a similar nature by Spiegler (21) on dogs and Dennig (22), (23) on man gave similar results. Landauer (18) explains this increased endogenous protein catabolism as a source of fluid for replacing some of the water removed while Spiegler (21) inteprets it as a result of tissue damage. In clinical conditions such as cholera where the dehydration and protein destruction is marked very high blood urea concentrations are reached (24). These increased blood urea concentrations as well as those found in certain dehydrated conditions of infants may easily be a result of increased urea formation due to the increased protein destruction which would follow and accompany such pronounced water losses.

Convulsions following the administration of water was one of our most interesting results in some of those rabbits which had been dehydrated with sucrose. The effects of water administration varied but usually resulted in a marked decrease in urine volume and urea excretion, if the animal lived long enough to allow us to obtain data on these points. Convulsions always took place within ten to fifteen minutes and often resulted in immediate death. That these were not caused by "water intoxication" (25) is proved by similar reactions to the administration of physiological saline as well as by the fact that the amounts of water taken were not excessively large. In a number of cases the blood urea presented a sharp rise following fluid ingestion. This might be explained as a washing out from the tissues of urea formed during the course of the experiment, which in turn suggests that the convulsions in our animals might be due to the washing out of some substance formed under the abnormal conditions, existing in the tissues, or of some other toxic principle evolved in

normal metabolism. The decrease in the urea ratio might also be due to such a substance. This phenomenon is being investigated further.

With the exception of one death found to be due to an acute lung infection, all of the rabbits which did not receive fluid simply became drowsy and quietly died.

SUMMARY

The effect on the blood urea concentration and on the rate of urea excretion of water abstinence and water loss has been studied in rabbits. Under the conditions of our experiments—simple abstinence from food and water, or complete starvation following the diuresis induced by intravenous injection of sucrose—the blood urea concentration gradually increased to many times the initial level.

The rate of urea excretion gradually increased in all cases. This increase was sometimes proportionately greater, more often less than the increase in blood urea concentration, so that on the average the ratio: $\frac{\text{Rate of urea excretion}}{\text{Blood urea concentration}}$ showed a slight decrease.

The urine urea concentration gradually rose but never attained any fixed and constant maximum.

The urine volume decreased early and then changed only slightly.

The increase in blood urea concentration in these experiments is ascribed mainly to an increased urea formation resulting from an accelerated tissue catabolism. The increased rate of urea excretion is the measure of this increase in urea formation. The mechanism under which the high level of blood urea concentration is attained is analogous to that which would result if gradually increasing amounts of urea could have been continuously injected into the animals.

Convulsions resulted from the administration of fluid (water or physiological saline) to animals which had remained long without food or water after preliminary dehydration by the intravenous injection of a sucrose solution.

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STUDIES ON THE EFFECTS OF BATHS ON MAN

I. Relationship between the Effects Produced and the Temperature of the Bath

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Baths have been used so generally in medical treatment that a very large literature exists as to their clinical use, but the literature dealing with the effects of baths on normal subjects is much less detailed. No attempt will be made here to give a complete list of references, but only a few which bear directly on the problem as to how far the effects produced by baths are determined by the changes in skin or body temperature.

James Currie (11) in 1797 used baths, both hot and cold, for treatment of fevers and observed mouth and axillary temperature. He noted that baths between 94° and 96°F. were neutral for himself and produced no effect on his body temperature, that baths 105°F. produced severe symptoms and if patients were given warm baths he advised they should be put into water at about 94° and that the temperature should then be gradually raised and not generally above 100°F.

A considerable amount of literature is in existence dealing with the effects of baths on the circulation; references to a number of these are given (9), (14), (23), (28). In general it has been found that hot baths lower blood pressure while cold baths raise it but Strasburger (30) pointed out that the results are complicated and that a cold bath in particular may produce an initial rise followed by a fall and again by a second rise. The same observer also noticed that hot baths above 40°C. often gave a blood pressure above normal, while with warm baths there was usually a fall of pressure; he found that a bath between 34 and 35°C. produced no change in blood pressure. Schapals (29) studied the circulation rate by respiratory methods and found no change in minute volume with baths from 34 to 35°, but a very much increased minute volume with very hot baths (which also gave considerable hyperpnea), the increase being produced by a fast pulse rate in spite of a diminished output per beat. On the other hand cold baths increased the minute output in spite of a slowing of the pulse. He found in hot baths that the increase in minute volume and circulation rate much exceeded the change in oxygen consumption. Similar

results to these were obtained by Lindhard (25) using a nitrous oxide method. On the other hand C. Tigerstedt (34) attributes most of the effects of baths to changes in venous return to the heart as the result of the hydrostatic pressure of the water and he found that varying the temperature of the water made little difference. In his experiments, however, anesthetised dogs were used, and the circulation was below normal as the result of the introduction of a stromuhr into the aorta, as was indicated by the low blood pressures recorded.

Recent experiments by Barcroft and Marshall (7) suggest that this contrast between the effects of different temperatures is real without at all excluding the additional mechanical factors of the water pressure suggested by Tigerstedt. Additional evidence of the effect on circulation of warm baths was obtained on anesthetised cats by Uyeno (35) which again indicated increased minute volume.

Hill and Flack (22) described the effect of hot baths of man showing a very marked fall in blood pressure and a very great hyperpnea and a considerable drop in alveolar CO_2 percentage. Quite recently Adolph and Fulton (3) have published figures on the effect of high room temperatures on the circulation.

That the respiratory effects of hot baths are mostly due to the change in body temperature is shown by the experiments of J. S. Haldane (20) and of Sutton (31) since similar effects were produced by hot rooms.

That the temperature is the main factor not only here but also in the production of some of the circulatory changes is shown by the work of Heymans (21) who produced in animals both hyperpnea and circulatory changes by heating the blood in an arteriovenous anastomosis. Heymans also showed that in such animals the CO_2 eliminated was increased by the rise in body temperature. Haggard (19) showed that hot baths produced a hyperpnea with a very considerable fall in alveolar CO_2 and that in the short period of his experiments (20 minutes) there was not a sufficient decrease in the alkali of the blood to compensate for this fall. Cajori, Crouter and Pemberton (10) showed with hot room experiments that there was enough change in acid-base equilibrium in quite mild exposure to heat to produce a measurable increase in the alkalinity of the blood.

Experiments on dogs by Flinn and Scott (15) have shown that a hyperpnea with an alkalosis can be produced by high room temperatures though the reaction is perhaps somewhat different, in degree at any rate, from those observed in man. Graham and Poulton (16) found that high temperatures produced in a warm room resulted in no increase in protein metabolism but they noted hyperpnea particularly in one subject, tingling sensations in the hands and an increase in the excretion of ammonia and acetone bodies on the day the high temperature was induced.

Warm baths have been shown by Barbour (5), (6) to produce a dilution of the blood in dogs in agreement with older literature including observations on man.

That temperature control is somewhat abnormal in a bath was indicated by the results obtained on patients treated for surgical wounds during the war by immersion in baths of running water up to the neck for periods extending over sometimes several months at the Eastern General Hospital, Cambridge, England. I have been unable to find any report of this treatment, but I am indebted to the surgeons in charge who showed me that these patients on recovery were uncomfortable unless the temperature of the water was sufficient to produce a mild pyrexia of about 37.5 and that even a normal subject kept in the bath for several days as a control was only comfortable when the water was adjusted so as to give him a similar slight pyrexia.

The effect of cold baths is dealt with in much of the above literature and is also reported at considerable length by Lefevre (24), so that our experiments have not attempted to deal with the effect of baths below a temperature of 34°.

A number of experiments have now been made on the effects of baths, at first in association with J. B. S. Haldane and later with various groups of students. It is proposed in this paper to amplify somewhat the brief report given in 1921 (8) and to give a general outline of the scope of the experiments, while later papers will deal in greater detail with the various points that arise. In this paper in particular an attempt will be made to put forward the evidence distinguishing the mechanical effects of the baths from those more definitely dependent on temperature.

Methods: The subjects were immersed up to the neck in a bath 5 feet long and with the water about 14 inches deep, provided with a stirrer keeping the water constantly circulating and the temperature of the bath was controlled by a thermostat. This bath was heated electrically in the earlier experiments; in the later by the introduction of hot water from a tank through a valve controlled magnetically by a thermostatic Hearson capsule and relay. In this way the temperature could be kept steady or could be altered 4°C. in either direction in as many minutes. For neutral temperatures the bath was kept between 35.5 and 36.5. In a bath of this size the subject is forced in order to be completely immersed to the neck, to assume a semi-reclining position with the knees bent and the pressure of water on the subject varies considerably in different areas. With a maximum water depth of 35 cm. the average depth of water over the abdomen was about 14 cm. and the average over the whole trunk and limbs would perhaps amount to 20 or 25 cm. of water.

The bath had a cover and except in very hot weather the subject wore a mackintosh cape so that there was no exposure of any part of the skin to

cold air except the head and neck. In the bath the subject always stood up and was exposed to the air for a short while when passing urine at each hourly interval. The body temperature was estimated in a few cases by rectal temperature measurements, occasionally using the recording resistance thermometer as described by Woodhead and Varrier-Jones (36). In most cases the temperature was taken by a clinical thermometer hung in the glass tube through which the urine was passed, which gave reliable readings unless the volume of urine was very small. In cases where the urinary temperature could not be readily determined mouth temperatures were employed. In all a total of 36 experiments have now been made on 14 subjects, including 4 women. Five of these subjects have been exposed as a control to high temperatures of moist air for comparison of the effects obtained, though in these experiments the observations have been much less detailed.

In the room experiments for the most part the subjects have sat naked in a room heated by steam escaping into the atmosphere, so that the air has usually been almost or completely saturated. As a rule the temperature of the room has varied between 36.5 and 37.5°. It is probable that this room, heated by steam reduced from a higher pressure introduces some complicating factors since there is a varying amount of fog and evidently particles of water are also present in the atmosphere. It is very noticeable that the air becomes very "heavy and oppressive" whenever the steam is let into the room by the thermo-regulator. Experiments on animals suggest that such a room air may actually cause some water-logging of the lung tissues, and so possibly interferes with pulmonary interchange; consequently little emphasis is laid on these room experiments here, since this factor has not yet received adequate investigation.

Estimations of arterial blood pressure have been made by the Riva Rocci method and auscultation. Alveolar air samples were taken by the Haldane method on J. B. S. H. only, since the mental confusion with baths of high temperature was sufficient to prevent accurate samples being obtained unless the subject was thoroughly accustomed to the process. Air samples have been taken on many subjects using either Douglas bags and valves or a spirometer, the latter being arranged in later experiments to give a graphic record. Gas analysis has been by the Haldane or Haldane-Henderson apparatus. The dead space of the Douglas valves has been 60 cc. and the alveolar carbon dioxide tension has been approximately computed by calculation allowing a total dead space of 200 cc.

Urines were titrated to phenolphthalein for total acidity, after the addition of neutral oxalate (Folin). Ammonia was determined sometimes by aeration and sometimes by Malfatti's method. The pH of the urine was determined colorimetrically by comparison with standards, precautions being taken to prevent loss of CO_2, (27) except in the initial observations

already briefly reported, where these precautions were not taken. Chlorides were estimated in many experiments by the Volhard or Volhard-Harvey method, ureas by Van Slyke's method, phosphates with uranium acetate, and in a few experiments qualitative tests were made for acetone and bicarbonate in the urine.

In a few experiments hemoglobin changes have been estimated using the method devised by Dreyer (13).

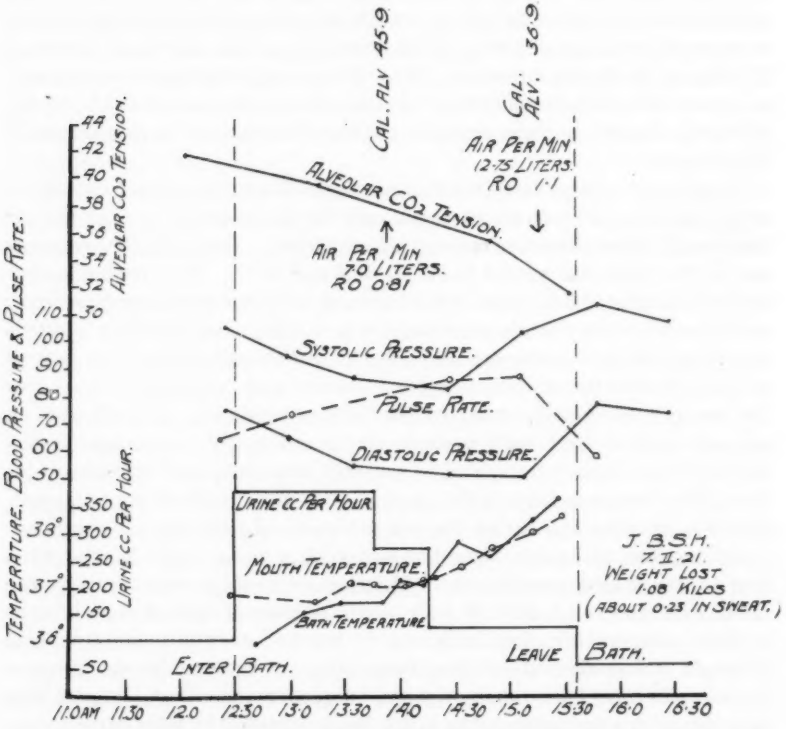

Fig. 1. Gradual rise of bath temperature on subject 1. Bath at 12:30 p.m. Breakfast ended about 3 hours earlier. Cal. alv. indicates CO_2 tension in alveolar air calculated from Douglas bag samples.

Results obtained: *Pulse rate.* Little or no change of pulse rate was noticed on changing from a lying down position outside the bath to a similar position in it, if the water was neutral in temperature. If the water was hot the rate rose and this rise was generally more or less proportional to the rise of body temperature. The similarity of these two curves may be seen in figures 1, 2 and 4 (mouth temperatures). That the

pulse rate was mainly determined by the temperature changes is shown by the fact that the same subject when tested in a hot room showed about the same change in pulse rate as in the bath for a given change in body temperature. Thus subject H. C. B. showed pulse rates of 121 and 112 for a

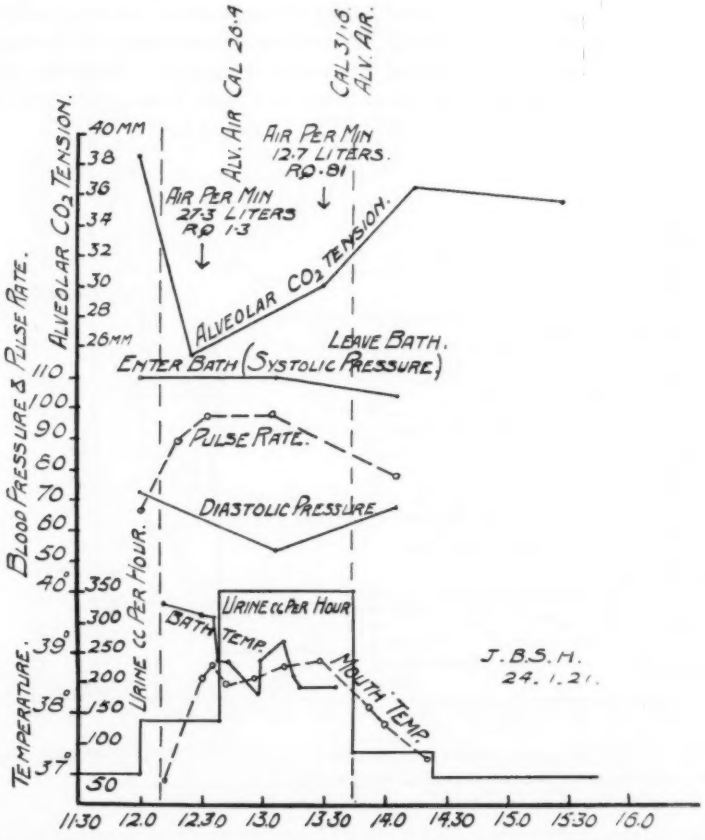

Fig. 2. High temperature bath on subject 1. Breakfast ended 2¼ hours earlier. Calculated alveolar air indicates alveolar CO_2 tension calculated from Douglas bag samples.

body temperature of 39.1 on two occasions in the bath and rates of 105 sitting at the same body temperature in a hot room on one occasion and on another 120 sitting and 98 lying down, although the rate at which his body temperature was changing varied considerably in the different experiments. In general there is a change in pulse rate of about 37 for a change

in body temperature of 2°C., that is to say, a change of about 10 beats for 1°F. (see table 1), so the results obtained resemble closely those seen in fever.

The temperature coefficient for such a change in pulse rate is about 9.5 so that the mechanism concerned is likely to be something much more complicated than the mere direct effect of temperature on heart muscle.

Occasionally in subject 1 (H. C. B.) changes in pulse rate near the end of an experiment were accompanied by severe symptoms. Thus the pulse rate changed in 5 minutes on one occasion in a bath from 120 to 140 with

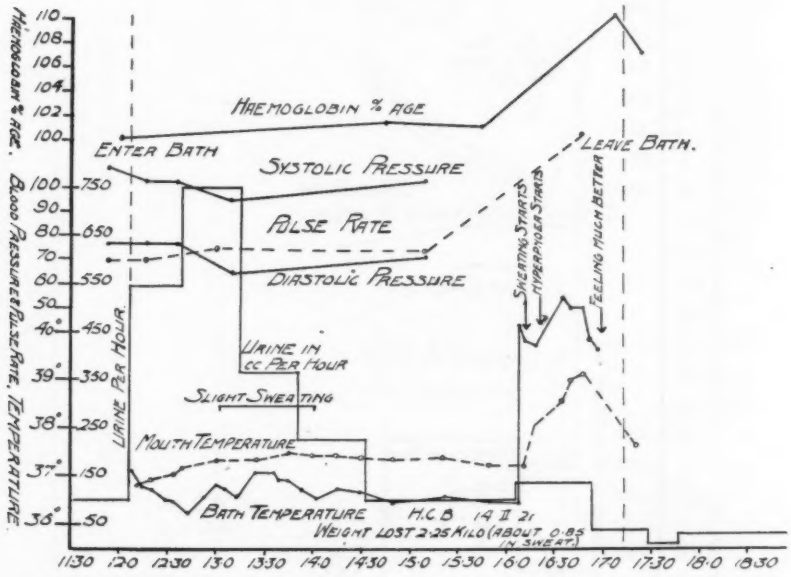

Fig. 3. Bath at nearly neutral temperature on subject 2 followed by later rise in bath temperature. Breakfast ended 3 hours earlier.

a rise of temperature from 39.4 to 40.0°C. and on another occasion rose from 108 to 120 with a body temperature rise from 38.2 to 38.8 in 34 minutes in a hot room, and on both occasions the subject (weight about 60 kilos) had lost over 2 kilos in weight in urine and sweat and appeared to be suffering from dehydration. The condition was characterized by a small feeble quick pulse. marked faintness, restlessness and an instinctive desire to raise the legs. Raising the lower limbs and the restless movements, both of which should increase venous return, caused some relief of the feeling of faintness.

These results are mostly in agreement with those recently reported by Adolph and Fulton (3) who also noticed a parallelism between the pulse rate and the mouth temperature, and a tendency to shock-like symptoms as the result of the exposure. These authors, however, considered the pulse rate dependent more on superficial than on deep temperature,

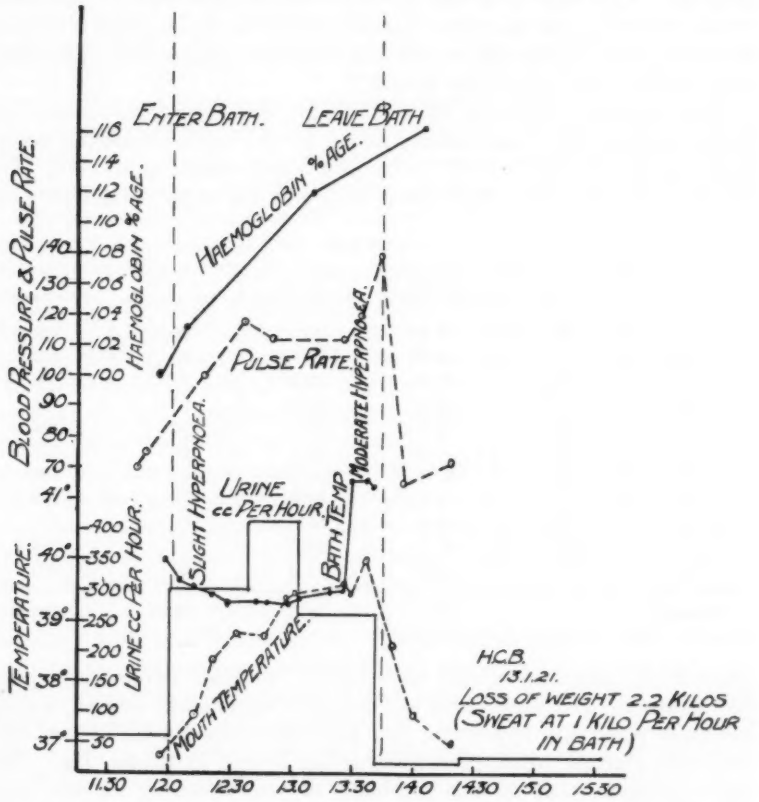

Fig. 4. Bath at high temperature on subject 2. Breakfast ended about 3 hours earlier.

since they found a greater correlation of the pulse rate with the mouth temperature than with the rectal temperature. While it is true that the rectal temperature is less upset by errors arising from external changes, it does not seem legitimate to assume that it is necessarily a truer indicator of body temperature than the mouth, when the body temperature is changing rapidly. The rectum has a relatively poor blood supply for

an organ of its mass, especially if it contains feces, and it is apt to show some considerable lag in temperature changes. In the bath the skin temperature is determined solely by that of the circulating water—with the exception of the head and neck—and it is clear from figures 2 and 4 that the pulse rate is not correlated at all closely, if at all, with the bath temperature. One must therefore conclude that the pulse rate is determined by the body temperature rather than skin temperature, though it is possible that the factor of importance is the subcutaneous temperature rather than that of either skin or internal tissues.

Blood pressure. Baths of 34° or 35°C. are said to produce no effect on blood pressure, but temperatures as low as this with circulating water, induce a definite fall in body temperature and sensations of cold. For the most part therefore, baths below 35.5 were not employed and for most

TABLE 1

NUMBER	SEX	INITIAL TEMPERATURE	FINAL TEMPERATURE	INITIAL PULSE RATE	FINAL PULSE RATE
1	M	37.0	39.4	70	120
1A		36.8	39.1	70	102
2	M	36.9	38.6	68	98
2A		36.9	38.0	65	86
3	M	36.9	38.9	80	104
4	M	37.0	39.1	68	102
5	M	37.4	39.3	72	120
5A		37.4	38.9	72	114
6	M	36.3	38.8	70	117
7	F	37.1	39.0	72	108
8	F	36.0	38.5	61	103
Averages.....		36.9	38.9	69.8	106.8

subjects temperatures of 36.0 were found "neutral" in relation to body temperature. But these baths felt definitely warm when entered, usually produced some skin hyperemia and generally caused a fall of both systolic and diastolic blood pressures varying from 0 to 10 mm. Hg. This fall of pressure may be only gradually developed during the first hour (see fig. 3). If the bath temperature is high the fall in diastolic pressure is usually greater, but the systolic pressure may fall somewhat (subject 1 (H. C. B.) about 6 mm.) or stay steady (subject 2 (J. B. S. H.) see fig. 2) or rise slightly (subject 3 (E. F. A.) 4 mm). If the body temperature rises considerably there is a tendency for a late rise in systolic pressure (fig. 1)and a preliminary fall of systolic pressure followed by a rise was not at all uncommon in hot baths. Symptoms of faintness may later supervene apparently with another fall of blood pressure, though at present only a few observations are available at this stage of the experiments.

A rise in body temperature resulting from the hot room similarly may cause either a fall or rise in systolic pressure though usually producing a considerable fall in diastolic pressure. Some as yet unpublished observations by some of my students suggest that there is considerable individual variation, though the character of the reaction of the same individual is relatively constant.

One must conclude therefore that the blood pressure changes in the baths are affected by temperature conditions, and that the fall of pressure in "neutral" baths may be due to a temperature of 36°C. being above the neutral point for skin temperature, though neutral for the maintenance of a normal body temperature. The circulatory changes with hot baths do not appear sufficient to be the primary cause of severe symptoms except after long continued dehydration from sweating. These conclusions are in agreement with those recently reached by Adolph and Fulton from hot room experiments except that they found a rise in the systolic pressure under these circumstances. The observations referred to above seem to indicate considerable individual variation in the type of response, and Adolph as a subject in a hot bath showed the same tendency to a rise in systolic pressure, giving a normal lying down value of 107/68 with a pulse rate of 80, a rise to 111/63 with a pulse rate of 108 after 25 minutes in a hot bath at 39.8°, dropping later to 95/45 with a pulse rate of 108 when sensations of faintness had supervened. I have never observed blood pressures as low as those reported by Hill and Flack (22) . It is probable that even for the same subject there may be variations in response according to the degree of high temperature to which he is exposed but at present there is no adequate reason to assume any different effect of hot rooms and hot baths in this respect. Further experiments on the circulatory changes in hot baths will be undertaken.

Loss of water in urine and sweat. A considerable diuresis has been seen in all baths. This is not for the most part dependent on the temperature. That cold, including cold baths, might produce a diuresis has long been known but reference to the results charted in figures 3 and 4 make it clear that there is a marked diuresis which only gradually reaches its maximum whether the temperature of the bath is high or neutral. The same subject immersed for 1 hour in a bath varying from 34.9 to 35.0° which felt cold, had a slight fall of body temperature after a preliminary slight rise, no appreciable change in pulse rate or blood pressure, but a rate of urinary secretion rising from 45 cc. to 472 cc. per hour; so that cool baths are also effective in producing diuresis. Another example of a low temperature experiment is charted in figure 5.

There is no evidence of any water absorption through the skin. The subject gains slightly in weight during the first 30 minutes; this increase amounts only to about 50 to 100 grams, and is probably due to the skin

becoming sodden. After this there is a loss of weight equalling that of the urine passed, unless the temperatures are high, when there is also loss of weight through sweating, in some subjects reaching a rate of 1.6 per cent of the body weight per hour.

The character of the diuresis in neutral baths will be given in more detail in the second paper of this series, so that emphasis will only be laid here on the variations seen as the result of varying the bath temperature. On the whole the rate of secretion of urine is less when loss of water through sweating is also going on, as might be expected—compare for instance figures 3 and 4. If the bath be prolonged at a neutral temperature the diuresis gradually subsides and returns to a normal level and the rate of secretion may then be again increased somewhat (see fig. 3) if the body temperature is raised. This second rise in rate appears to be determined by the necessity of excreting alkali under these conditions, for this urine contained large amounts of sodium bicarbonate.

No great differences between the urines excreted at different temperatures has been distinguished, except in relation to the acidity. With baths at high temperatures there is a definite hyperpnea, to be described later, and it is not surprising that the urine shows a great increase in pH and has a considerable decrease in titratable acid and ammonia. It is possible however that even slightly raised body temperatures, as in the earlier part of the experiment charted in figure 3, may cause a decrease in the titratable acidity. Figure 5 shows the rate of urinary secretion and of excretion of titratable acid and ammonia in subject 1 in baths at different temperatures. It will be noticed that with a bath at 35° in which the body temperature fell somewhat, the diuresis caused some increase in the acid excretion, while a bath of 36.5 gave a slight rise of temperature and a slight fall of acid excretion, this fall being much exaggerated when the rate of rise in temperature was later increased, while in the bath at temperature of 39.5 there was an immediate and rapid fall of titratable acidity. In the experiment charted in figure 5 A the conditions were not the same as for B and C, but with baths at slightly higher temperatures on this same subject the total acidity was decreased whatever other conditions existed.

On the other hand the body temperature can hardly be the only factor as is suggested by this chart, since other experiments on various subjects give either moderate increases or decreases in the total acid excreted (acid plus ammonia) even without any definite body temperature change. Any real rise of body temperature however is always associated with a decreased acid excretion.

The effects of the baths and the resulting diuresis on the urinary pH are somewhat different. In all experiments the pH changed to the alkaline side and usually was between 6.8 and 7.5 even when the titratable acid excreted per hour was not decreased, though with the diuresis the titrat-

able acidity per cent was lower. If the bath temperatures were high there was however a much greater alkalinity and the urines reached pH values of 7.8 even when loss of CO_2 was prevented.

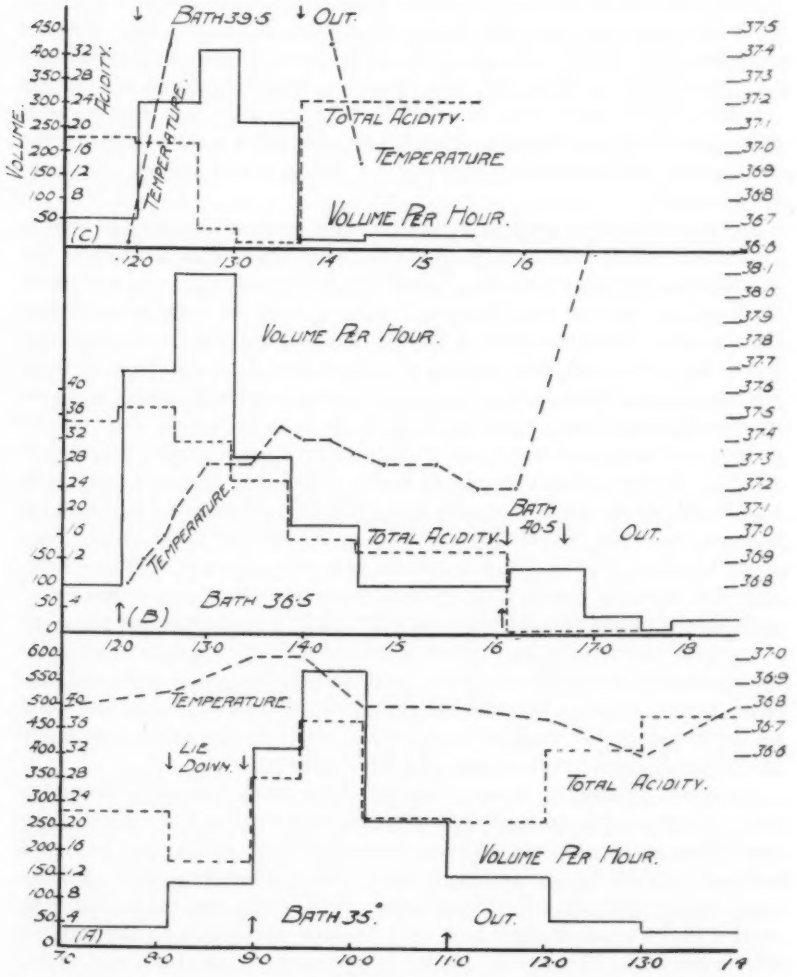

Fig. 5. Contrast between effects of baths at different temperature on subject 2. Total acidity curves gives sum of titratable acidity and ammonia (Malfatti) per hour in milliequivalents. Curve *A*—No breakfast but ½ slice bread and butter hourly from 7 a.m. Curves *B* and *C* present same experiments as figures 3 and 4.

In the experiments in a hot room when the body temperature rose slowly the urinary changes were very different. There was no marked diuresis, though usually there was at first a slight increase in the rate of excretion. Thus in subject 1, entering a room kept at about 37.5 dry bulb and about 33° wet bulb raised the rate of urinary secretion from 48 to 65 cc. per hour in the first hour, but after this the rate dropped to 21 cc. per hour. The hot room has little effect on the pH of the urine there being sometimes a slight rise, sometimes a slight fall, the changes amounting to about 0.2 pH (similar changes have been observed by Talbert (33)), but the titratable acidity per hour was usually somewhat diminished, a result similar to the diminished acid excretion produced by baths which raised the body temperature.

It seems therefore possible to state definitely that the diuresis seen in baths is an effect produced by the bath, more or less independent of the temperature and that with this diuresis the pH tends to approach neutrality, but may become very alkaline if there is a rise in body temperature. On the other hand the bath of neutral temperature produces slight and inconstant changes in the amount of acid excreted per hour, but if there is a rise of body temperature, the acidity is considerably diminished. Loss of water through sweat occurs as freely in the bath with high temperatures as in a hot room and the loss is indicated by a considerable decrease in weight. In the preliminary report it was stated that there was no loss of acid in the sweat, since this had a pH of 7.9; this determination was made however on sweat collected from the face, which had undoubtedly been altered by loss of CO_2. In a few of the later experiments sweat has been collected from the hands after careful washing without exposure to air;[1] under these conditions pH values of 6.0 to 6.7 were obtained at the commencement of sweating and values of 6.5 to 6.9 thirty minutes later (compare Adolph (2) and Talbert (33)). It is not possible to say at present how much acid is excreted through the skin under the conditions of the bath, but judging by other work on the excretion of CO_2 by the skin it is unlikely that there is any important loss of acid by this path (4).

Respiratory changes. A very marked hyperpnea gradually becoming more severe until it amounted to dyspnea, was seen in hot baths. The ventilation rate was increased enormously mostly by an increase in depth and only slightly by an increase in rate. With some subjects it came on very rapidly soon after the temperature of the water was raised but with most it had a gradual onset and only became noticeable to the subject after some 10 to 15 minutes. If the temperature of the bath was maintained at a constant level the maximum hyperpnea would then be developed at a time when the sensation of high skin temperature was beginning to disappear; it is not therefore easily explained by skin stimu-

[1] According to a method suggested by Dr. M. H. Jacobs.

lation alone. The hyperpnea increased in amount during an experiment even when the gradient of the temperature rise remained relatively constant. It cannot therefore be simply dependent on the gradient. On the other hand it will be seen from figures 1 and 2 that subject J. B. S. H. had the same degree of hypernea with a temperature of 38.3 rising at about 1.05° per hour as at a temperature of 38.9 maintained at a steady level, and in both cases it was much less than occurred with a temperature of about 38° rising at 5° per hour, so that body temperature level does not appear to be the main factor.

In hot rooms there is also some hyperpnea, but it is much less noticeable. In the room however the rate of rise of temperature has usually been 1.3 to 1.5° per hour and it has never exceeded 1.9° per hour. In baths a common rate of change was 4 or 5° per hour and rates of increase of even 8° per hour have been observed for short periods.

The hyperpnea observed and its variations may be accounted for tentatively by assuming that it is an effect produced on the respiratory center by stimulation of the skin by heat, the sensitivity of the center to such stimulation varying with its temperature at the time.

With the hyperpnea there is a fall in the alveolar CO_2 tension (see figs. 1 and 2). As has been already explained direct measurements of this were only made on the trained subject J. B. S. H. On this subject respiratory samples were also taken with Douglas bags and from these an approximate alveolar CO_2 tension has been computed, estimating the dead space of the subject as 140 cc. In this way it is possible to compare such computed figures with those found by direct measurement a short time earlier or later in the experiment. The two methods gave approximately the same figures. Thus on one occasion, that plotted in fig. 2, calculation from the respiratory sample taken at 13.37 gave a CO_2 tension of 31.6 mm. direct measurement 6 minutes earlier having given 30.1; on another (that plotted in fig. 1) the respiratory sample taken at 15.22 gave 36.9 mm. direct measurement 8 minutes later giving 31.2. These two examples represent the occasions when there was the best and the worst agreement of the series. There was perhaps a variable error in the calculated figures as the result of changes in dead space resulting from varying depth of breathing (12), but the deeper breathing only gave a tidal air of 788 in this subject. From these results it would appear legitimate to use calculated figures as approximate values, and this is done in considering the results of later experiments.

Details of the respiratory changes obtained will be given in the third paper of this series in connection with the observed changes in the pH of the blood and consequently no further figures will be included here.

Hemoglobin changes. An increase in the hemoglobin percentage has been observed in the hot baths when there has been extreme and continued

sweating (see figs. 3 and 4). These hemoglobin changes were observed in subject H. C. B. whose normal diurnal variations in hemoglobin had been tested, and no such change was seen when the hot bath was not taken. In this subject even a bath at neutral temperature has always produced a slight increase in hemoglobin percentage but in other subjects neutral temperature baths have produced an initial slight dilution followed by a slight concentration and some further figures on the effects of neutral temperature baths are included in the following paper. It seems unlikely that the mechanical effect of the water can be entirely neglected and that changes obtained in hot baths can necessarily be ascribed entirely to temperature conditions, so that it is not certain that the slight concentration of hemoglobin followed later by a considerable change shown in figures 3 and 4 would have been seen under the conditions of a hot room.

The only statement that is warranted at present is that these experiments give insufficient data to determine the effect of sweating on blood concentration, since other factors have also been present.* Under these circumstances it is unfortunate that some of the figures I have obtained have been quoted by Adolph (1) as applying to simple dehydration by sweating and have been requoted by Marriott (26). The views there expressed may be true but are not established at present.

Temperature sensations. Baths at 35° are above the normal skin temperature of the body and feel warm when entered. After a short while they feel quite comfortable and then usually feel somewhat cooler than the subject would choose. Usually there is little change in body temperature. There seems to be no doubt that if guided by his own sensations alone the subject would choose a bath temperature at a slightly higher temperature and one sufficiently high to give him a small rise in body temperature.

If the temperature of the bath is raised quickly, it feels very hot, the sensation of heat gradually diminishing. If the bath be cooled to a temperature of 38° when the subject's temperature is 39 it feels comfortably cool. If the bath be cooled to 32 or 33° when the subject's temperature is 39° a very definite sensation of chilliness is induced with a tendency to shiver, and actual shivering may be caused even though the body temperature is still much above normal (even above 39°). A similar shivering with an abnormally high body temperature has been observed after a hot room experiment on coming into a room at 13.8°.

This therefore affords further evidence of the importance either of the skin or subcutaneous temperature in temperature control.

A single experiment has been performed on subject H. C. B. when he had a pyrexia of 37.3 at the commencement of an influenzal attack. A much higher temperature bath was necessary to induce sweating under these circumstances than normally, sweating not commencing until the mouth temperature reached slightly above 38°—some 0.5° to 0.7° higher

* Recently, however, Flinn has demonstrated such a blood concentration in the dog. This Journal, 1924, lxx, 194.

than usual. The body temperature was raised in the bath to 39.5, rose on leaving the bath to 40° and maintained this high level for several days.

Symptoms. No symptoms are produced by baths at neutral temperature. With baths with circulating water at 38° to 40° besides the dyspnea there occurs often considerable palpitation and the pulsation of the vessels in the extremities is often very noticeable and unpleasant. Later mental symptoms may come on; the subject is then very excitable, rapidly loses his temper, may not be able to think of the right words to express himself and may feel faint, or may even actually faint. These mental symptoms are relieved by inhaling oxygen from a Douglas bag, are also relieved by breathing expired air with a relatively high CO_2 content and low oxygen content, but are exaggerated if ordinary air is breathed through valves from a Douglas bag. Consequently the improvement produced by the other gas mixtures is produced in spite of the fact that the valves themselves seem to increase the discomfort of the subject. A typical result of this sort was reported in the preliminary communication, similar results have since been often obtained on students, when the subject was ignorant of the mixture administered. The causation of the improvement has not yet been analyzed. The view suggested in the preliminary report that an alkalosis occurs sufficient to make oxyhemaglobin stable may be true in the arteries but is not supported by actual pH determinations on blood samples from arm veins, which are reported in the third paper of this series.

On a number of occasions tetany has been seen, starting usually with typical tonic spasms of the muscles of the hand and spreading to muscles in the legs, thighs and abdomen. At first there is merely a tendency to assume these tonic positions, it being still possible for the subject to relax the muscles with a considerable mental effort. After a time the condition progresses and relaxation is no longer possible. At this stage the very great desire for deep dyspneic breaths coupled with the hampering effect of strong tonic contractions in the abdominal muscles, which are difficult to relax, causes an intensely unpleasant and frightening sensation in the subject. Possibly the condition is dangerous. Our preliminary report was taken by Greenwald (18) as implying that the tetany was relieved by breathing oxygen. This we were careful not to state, and further work described in the third paper of the series shows definitely that this is not the case, our results therefore are in agreement with those of Grant (17).

I, myself, have acted as subject for the hot baths much more than any other subject, though for the most part these experimental baths have been at long intervals apart, extending over many years. It is now noticeable that much higher temperatures have to be used in the baths to produce severe symptoms in me than in other subjects, and also higher tempera-

tures have to be used than were needed for me previously. The causation of this change however is not definite since the climatic conditions under which I have been living have also been changed during this period.

It can, however, be stated that in me the symptoms of mental confusion and of tetany have not appeared to have a common origin. If the rate of temperature rise is very fast and the experiment consequently of short duration, tetany is readily induced but mental symptoms are only moderately in evidence; with a slower but more prolonged experiment mental symptoms are more definite, and tetany only slight; with a yet slower rate of change neither mental symptoms nor tetany are seen but faintness comes on after a certain stage of dehydration. These remarks on symptoms are somewhat difficult to prove, but are the impressions resulting from 9 experiments in hot baths and 3 in hot rooms on myself.

It should perhaps be noted that considerable differences are produced by the circulation of the water. The symptoms produced by baths at 39.5 or 40° with circulating water compare more or less with those produced by a bath at 42° or higher, in which the water is not kept constantly stirred.

CONCLUSIONS

1. The general changes observed in man as the result of immersion in a hot or warm bath are reviewed and an attempt is made to distinguish the mechanical effects of the bath from those due to temperature. Further details of particular responses will be included in later papers.

2. The circulatory and respiratory changes observed are concluded to be dependent mainly on changes both in body and skin temperature.

3. A diuresis of considerable degree is observed and is relatively independent of the temperature of the bath, but the amount of titratable acid excreted per hour is much influenced by changes in body temperature.

4. A summary is given of the symptoms so far observed during the experiments.

It is a pleasure to express my thanks to Dr. J. B. S. Haldane who coöperated in the earlier part of this work, and to many of my students who have assisted in later experiments.

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STUDIES ON THE EFFECTS OF BATHS ON MAN

II. The Diuresis Caused by Warm Baths, Together with some Observations on Urinary Tides

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The occurrence of a diuresis in baths was first reported briefly by one of us (H. C. B.) in conjunction with J. B. S. Haldane in 1921 (2). Further details have since been given which show that this diuresis is caused by the bath and is not dependent on mere temperature changes (3). The experiments here reported were designed to give further information as to the character of this diuresis.

As a control some analyses have been made of hourly samples of urine obtained under the same conditions, but without the bath. By examination of the control figures it was apparent that certain urinary tides were to be found to a varying extent in different individuals and in order to decide if the same factors were dominant in producing these tidal conditions and the diuresis seen in baths, some further control observations were made. These showed quite clearly that in most subjects there is a definite chloride tide on waking in the morning with an increase not only in the total quantity excreted per hour but often also an actual increase in the percentage strength. We recognize that these control experiments are incomplete as a study of urinary tides, but they were not undertaken for that purpose.

The occurrence of urinary tides of various types has been known for many years. An alkaline tide after meals was described by Bence Jones in 1845 (4) and has been investigated more recently by Hasselbach (13) and by Fiske (11). The occurrence of an alkaline tide on waking and independent of meals has been described by Leathes (16) and by Endres (10) and more recently Watson (29) has also found it to be dependent on waking rather than food. It will be seen that we can entirely confirm the occurrence of a considerable alkaline tide on waking even when all other factors are kept constant, though this is more marked in some subjects than in others.

A phosphate tide (with a low level in the early morning) was described by Beneke in 1854 (5) and was noted by Cathcart, Kennaway and Leathes (8) in conjunction with a uric acid tide in 1907 and further observa-

tions on it have been reported by Broadhurst and Leathers in 1920 (6) and Fiske in 1921 (12).

Comparisons of day and night urine have been made by many workers and recently by Campbell and Webster (7); they found the chloride excretion to be lower at night than during the day and the phosphate and acid excretion to be more or less proportional to one another. In an excellent study of sleeplessness in man by Kleitman (15) some urine observations were included. He found the excretion of acid and phosphates to be higher at night than during the day and the chloride excretion to be less at night; on a reverse routine in which the time of sleep was altered but the meals were left as before he found a reversal of the differences in rate of acid and phosphate excretion and a tendency to reversal in the chloride excretion.

There is therefore evidence in the literature suggesting the presence of a chloride tide though this has never to our knowledge been investigated on hourly samples of urine.

METHODS EMPLOYED: In the case of the experiments conducted with J. B. S. Haldane breakfast was taken as usual, and the bath was entered about midday two or three hours after the end of that meal, and no further food or drink was taken till the end of the experiment. In some later experiments on students, baths have been taken at 10:30 a.m. after a breakfast at 7 a.m. limited to 1 slice of bread and butter and 1 cup of fluid, while in the more detailed experiments the subject woke up at 5 a.m., lay in bed till 6 a.m., when he got up, and from 7 a.m. till the end of the experiment he took a meal consisting of half a slice of bread and butter and 100 cc. of water hourly immediately after passing urine. If a bath was included in the experiment it commenced between 9 and 11 a.m. The reason for the adoption of this routine was to keep the effects of meals as constant as possible; they could not be entirely omitted because the experiments were mainly conducted during the summer months and it would not have been possible to get adequate hourly samples of urine in the control periods, if no fluid were taken. These more detailed observations have mostly been made by the women authors on one another, though some experiments have also been made on H. C. B. under these conditions for comparison with the other figures.

The methods of analysis used were the same as those mentioned in the previous paper (3). In the control experiments when urinary samples were collected during the night or early morning loss of CO_2 during transport to the laboratory was difficult to avoid, and, as these were mostly concentrated acid urines in which CO_2 was relatively unimportant, no precautions were taken to prevent its loss. When pH figures are given for urines in which loss of CO_2 has occurred freely, attention is drawn to this fact, or such figures are inserted in brackets, In all other cases the precau-

tions suggested by Marshall (17) were taken to prevent loss of CO_2. With female subjects some loss of CO_2 is probably almost inevitable, but such loss was diminished as much as possible by using small long stemmed funnels dipping under paraffin oil, and there was at any rate only a partial loss of CO_2, since aeration of such urines still produced a considerable increase in alkalinity.

The bath has already been described It was kept usually at about 36°C., invariably between 35° and 37°. A few experiments were made on the effect of partial immersion, in which the subject sat in the bath on boxes of different heights, so determining the degree of immersion. Under such conditions a mackintosh cape was always worn and a cover was kept over the bath, so that only warm moist air circulated round the body.

Results obtained: control experiments and urinary tides. In most of our experiments the subject has passed urine immediately on waking at 5 a.m. (in the male subject without getting out of bed) and has then followed the routine already described. In three experiments the subject has continued in bed a second hour before getting up. Food has generally been taken two hours after waking (but has been postponed to three hours in three experiments) and has then been continued at hourly intervals as already described. After 9 o'clock the subject has either entered the bath or has continued the routine hourly meal until mid-day or early afternoon. In a few experiments the subject has awakened in the middle of the night and passed urine, so that a comparison could be made between the urine secreted in the earlier and that excreted in the later part of the night.

Observations on the urinary changes in the first few hours after waking mainly under this routine but with some modifications in certain cases have been made in 16 experiments on four different subjects; hourly observations on the urine have been continued later without taking a bath in six of these experiments on three subjects. Experiments in which the night urine has been divided into two parts have been made on six occasions on three subjects.

There is so much variation in the urinary changes in different subjects that it is difficult to collect the results into any one table, especially as even same subject will show variations from day to day. The subjects happened to show very considerably contrasts; thus subject 3 (W. S.) gave a urinary secretion usually of only 11 to 24 cc. per hour in the night and early morning and even when taking hourly 100 cc. of water the volume of urine rarely rose much above this level even after six hours of this routine. At the other extreme subject 1 (C.C.) would often secrete during the night at the rate of about 50 to 150 cc. per hour and this might rise in the morning on waking without taking food or drink as high as 200 cc. an hour. In this subject and also in subject 2 (H. C. B.) the rate of secretion

of urine was very variable and even slight changes in room temperature might produce enormous effects on the volume excreted per hour, while in the case of subject 3 (W. S.) the rate of excretion in the control period was relatively constant and not easily affected by the climatic conditions.

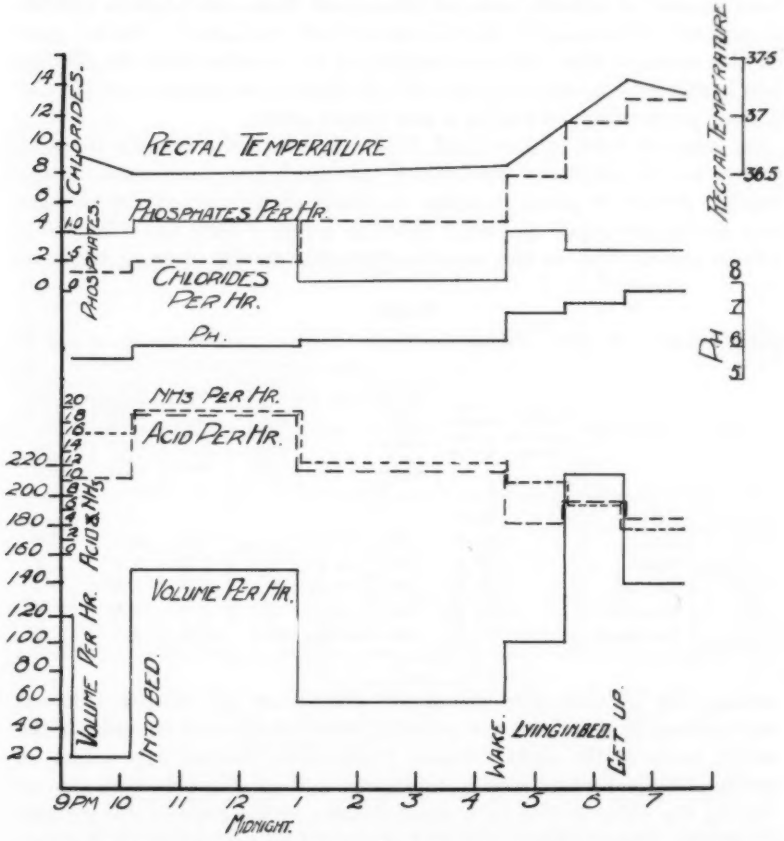

Fig. 1. Subject 1, September 12, 1923. Urine secretion during night and early morning. No food or drink taken from 6:40 p.m. previous evening. Chart shows in this and succeeding figures volume of urine per hour in cubic centimeters, titratable acidity, ammonia (Malfatti), and chlorides in milliequivalents, phosphates in millimols per hour. pH values obtained after loss of CO_2.

Under these circumstances it will be easiest to give several typical experiments on different subjects in whom the changes are seen either at their maximum or minimum, and then to compare briefly the results obtained in general.

An example from subject 1 in whom the alkaline tide is very marked although no food was taken is given in figure 1. On this occasion there was a big rise in body temperature during the first two hours after waking and with this a considerable increase in the alkalinity and an increase in the total amount of chloride excreted throughout these two hours in bed, although the percentage of chloride was at first diminished. Table 1 gives another example from the same subject on an occasion when the alkaline tide is still very marked but the chloride tide is not present and the rise of body temperature on waking is also almost absent.

An example from subject 2 (H. C. B.) in whom the chloride tide was always very marked and the alkaline tide much less conspicuous though usually present is given in figure 2, which shows the urinary changes seen during the night and early morning before a bath was entered. It will be noticed that on this occasion there was no rise of temperature on

TABLE 1

Date September 23, 1923. Subject 1. Dinner finished previous evening at 7 p.m.

TIME	CONDITION	ROOM OR BATH TEMPERATURE	BODY TEMPERATURE RECTAL	VOLUME URINE PER HOUR IN PREVIOUS PERIOD	pH	TITRATABLE ACID IN MILLIEQUIVALENTS PER HOUR	NH_3 IN MILLIEQUIVALENTS PER HOUR	CHLORIDES IN MILLIEQUIVALENTS PER HOUR	PHOSPHATES IN MILLIMOLS PER HOUR
9 p.m.	Urine		36.85						
10:20	Bed		36,45	33	(5.2)	4.4	5.9	2.0	0.4
4:45 a.m.	Wake		36.45	98	(6.4)	6.6	7.7	8.9	1.5
5:45	Get up		36.50	97	(7.0)	1.3	1.7	8.1	1.2
6:45	1st meal		36.70	193	(7.2)	2.7	1.9	8.3	0.8
7:45	2nd meal		37.20	245	(5.8)	14.9	42.2	4.9	2.7

waking, the alkaline tide was almost absent but the chloride tide was very noticeable and there was possibly some retention of chlorides in the earlier hours of the night. Figure 3 represents another experiment on the same subject where a temperature rise and alkaline tide were seen on waking, the chloride tide being again evident with a considerable increase in chloride concentration. Figure 4 shows a third experiment on this subject; it shows more clearly results similar to those of figure 2 and also an interesting contrast between the changes in chloride and phosphate excretion, the chloride excretion increasing when that of the phosphate diminishes. The effects of the previous meal may have complicated the earlier urines, but can perhaps be left out of consideration by 12:52 a.m., five and one-half hours after the end of the meal. Similar contrasts between chloride and phosphate excretion have been noticed in other experiments but less definitely, the earlier part of figure 2 providing an example.

In the experiment plotted in figure 4 the subject lay down at 9:15 a.m. and this was followed by a considerable diuresis. Such a diuresis produced by lying down has always been detectable in our experiments,

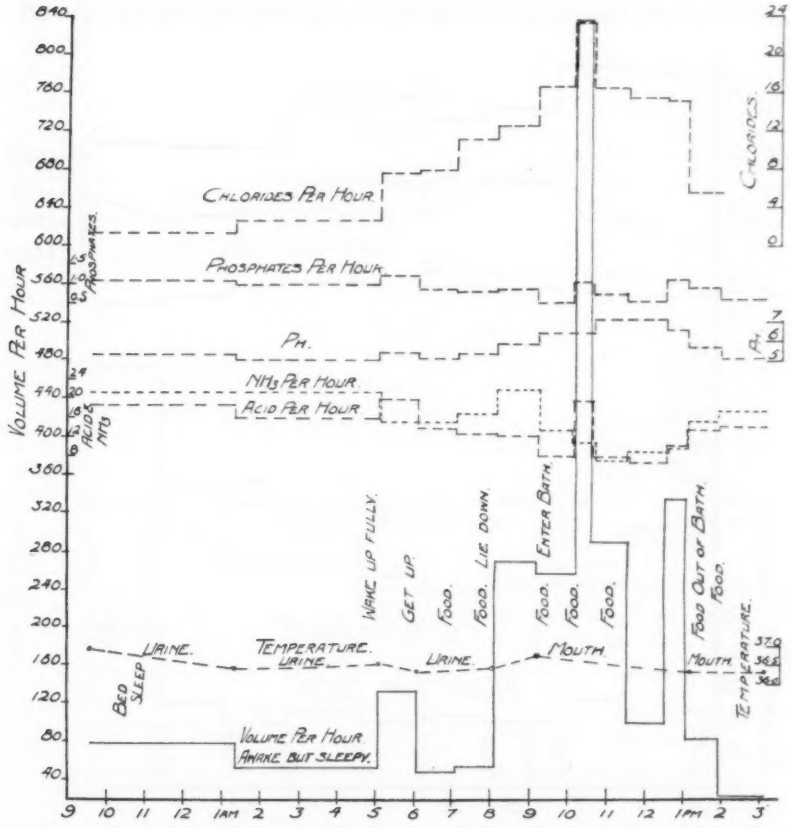

Fig. 2. Subject 2, July 31, 1923. Urine secretion throughout night and morning. pH values with loss of CO_2 in first 4 samples. Dinner finished at 7 p.m. Water 200 cc. at 10:26 p.m. Food and water hourly as usual from 7 a.m. Room temperature 17°C. (much below average for August 1) and subject felt cold while lying down. Urine taken by a syphon arrangement without getting out of bath and difficulty in completely emptying bladder was probable cause of apparent irregular rate of secretion during bath.

but is rarely so great. On this occasion the room temperature was 20.3°C. (relatively low for this climate in the summer) and the subject felt very chilly while lying down and the diuresis was probably partly due

to cold. Every diuresis that we have observed whether sitting, lying or moving about in which cold has been apparently a principal factor has always shown a considerable increase in the water excreted with an increase in chlorides, little or no change in total phosphates and variable effects on urea excretion. On the other hand when cold can be excluded

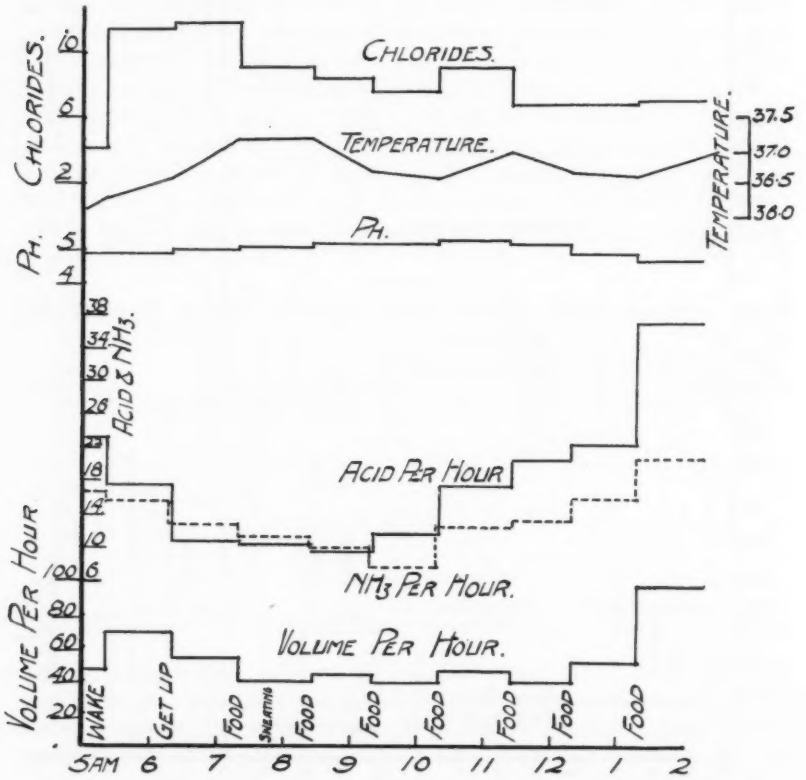

Fig. 3. Subject 2, July 15, 1923. Urine secretion throughout day without bath. Urine temperatures. pH values with loss of CO_2 up to 7:20 a.m., without loss of CO_2 after this time. Room temperature 22.5°C., some sweating. Breeze and no perceptible sweating after 12:00. Water 100 cc. and ½ slice of bread and butter taken hourly from 7:20 a.m.

there has been still a diuresis while lying down and this has been accompanied by a rise in the urea excreted per hour and sometimes even by an increase in the urea percentage. An increase in water and chloride excretion on lying down is also seen in figure 2, but this experiment was also conducted on an unusually cold day in August when the room temperature was only

17° and the subject again felt very cold. Instances of such a diuresis when cold apparently could be excluded are seen in figures 5, 6 and 8. The diuresis which may be seen on lying down is probably related to that produced by baths and it will therefore be considered further a little later.

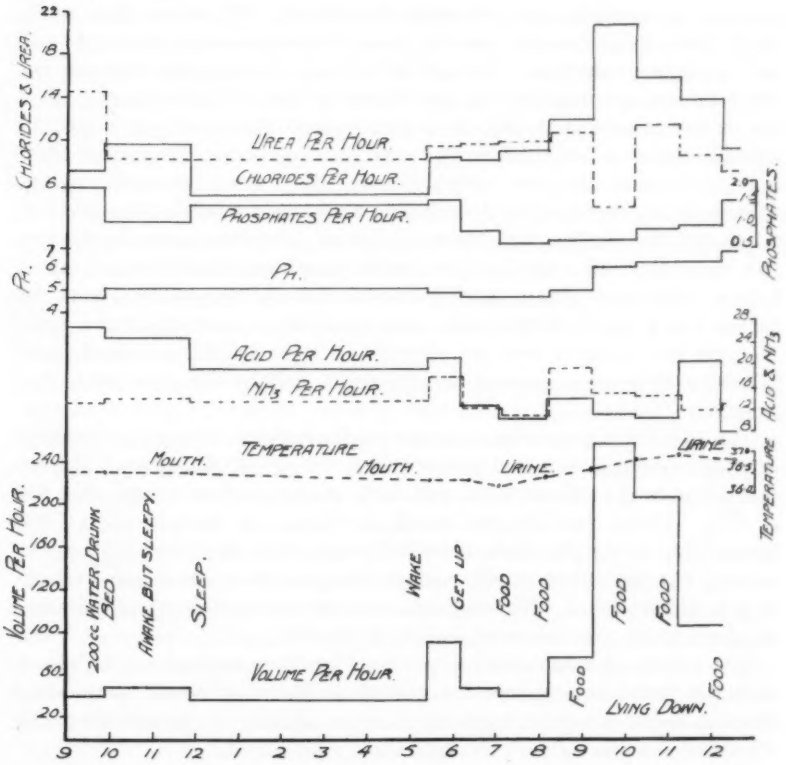

Fig. 4. Subject 2, August 8, 1923. Urine secretion during night and morning, effect of lying down without bath. pH values till 8:13 with loss of CO_2. Urea excretion charted in millimols per hour. Malfatti ammonia figures charted, though aeration figures also obtained. Dinner finished at 6:35 p.m. Water 200 cc. at 9:51 p.m. and usual hourly meal from 7:10 a.m. Room temperature 20.3°C.

General effects of waking and going to sleep. On waking in general a decrease in the titratable acidity usually occurs with, but sometimes without, an increase in pH; accompanying this there was an increase in the water and chloride excreted per hour, some diminution in the phosphate excretion, this last often lagging a little behind the other changes, and at the

same time there was a rise in body temperature, even though the subject lay as quietly as possible in bed. These various changes may be related to one another. The common inverse relationship of phosphate and chloride excretion has been already referred to and is readily seen on the charts, but an increased chloride secretion from the effect of cold, lying down or a bath is not accompanied by phosphate retention. The charts also demonstrate how closely parallel may be the curves representing titratable acid and phosphate excretion. Indication of some relationship between the other factors are supplied by the following data. A considerable alkaline tide was seen on waking in all four experiments on subject 1 but the chloride tide and temperature rise were only present in three out of these four experiments and were both absent together on one occasion. On the other hand in subject 2 the chloride tide was present in all four experiments made, but the alkaline tide and temperature rise were absent together on two occasions. In a third subject in six experiments the chloride and alkaline tides were always both present but body temperatures were not taken. In a fourth subject only two experiments were made and again no body temperatures were recorded; in one experiment both alkaline and chlorides tides were present though slight, and in the other both were absent.

The alkaline tide referred to above applies solely to changes in titratable acid and ammonia excreted per hour and not to pH changes, which were not determined on these night and early morning urines except after loss of CO_2. There was however usually a change of the pH value (after loss of CO_2) to the alkaline side with this morning tide (9 out of 16 experiments), but there was no appreciable change on 3 occasions and a change to the acid side on 4. By comparison the titratable acidity was decreased in 13 out of 16 , and increased in only 3 out of 16.

The volume of urine excreted per hour was increased on waking also in 13 out of the 16 experiments, but, though no doubt influenced by the other tides, it has no absolute dependence on the alkaline or chloride tide, since it has been observed even when both these were absent.

There is therefore probably some relationship between chloride, acid and phosphate excretions and between all these and changes in body temperature (compare effects of baths at different temperatures) (3) but no one factor appears to be always associated with any other invariably, though in any one subject definite rules appear to be obeyed.

The observations made on the effects of going to sleep are too few at present to deserve more than brief mention for comparison with the morning changes, since the effects have been complicated by two other factors, lying down and the proximity of the evening meal, which probably both affect urinary secretion at night and we have not differentiated the effects of sleep from these. Our 6 experiments must, however, be referred to

since they do give some evidence that the onset of sleep causes the opposite changes to those of waking and in particular retention of chlorides. Thus in the experiment charted in figure 4, subject 2 did not go to sleep for some hours after getting to bed; the volume of urine and total chlorides were increased after getting to bed but were diminished with the onset of sleep. Sleep produced no increase in acidity however, but the acid and alkaline tides have not been marked in this subject except accompanying body temperature changes, which on this occasion were very slight.

On the other hand in this same subject the experiment plotted in figure 2 showed an increase in the chloride excretion in the later part of the night associated with a half-awake condition, and a slight rise in temperature.

These results may be compared with those in subject 1 plotted in figure 1 in which simultaneous going to bed and falling to sleep caused a slight increase in the chloride and a considerable increase in the water and acid excretion. The apparent absence of any chloride retention with onset of sleep is probably to be explained by the opposing effect of lying down, an effect described a little later in this paper.

In obtaining further data it will have to be recognized that various degrees of sleep may exist. Thus in figure 2 a definite chloride tide commenced at 5 a.m. when the subject endeavored to wake up fully, though he had been partly awake for several hours and this happened in spite of every precaution being taken to avoid any activity at this time, the subject remaining quietly in bed throughout the next hour.

For the present the data suggest that the effect of going to sleep on urinary excretion is the opposite of waking. There is no evidence however that the increased acid excretion at night and the morning alkaline tide, which have been so long known, depend essentially on the diurnal changes in body temperature, although such a relationship might have been anticipated from the effect of baths at different temperatures on acid excretion (3), and although probably such body temperature changes are not negligible.

Lying down. The rate of urine formation may be increased by lying down, but this is no new observation. Volhard (28) has mentioned its occurrence and Neukirch and Neuhaus (19) have described it as particularly in evidence in asthenic individuals. Possibly this reaction has not only complicated our own figures in the comparison of night and day urines but also accounted for the somewhat variable relationship of night to day urine described by Oesterberg and Wolff (20). In any case the increased rate of secretion has always been evident in our experiments, especially soon after lying down, and is therefore made much clearer when urines are collected at short intervals.

Two charts (figs. 2 and 4) have already been given showing a diuresis in subject 2 on lying down but in both of these the experiment was complicated by sensations of cold. In the experiment shown in figure 4 this diuresis on lying down was accompanied at first by a diminution in the urea excreted, later by a slight rise. This was an exception to the changes usually found. On two other occasions also with subject 2 the urea excretion was considerably increased on lying down and this was also the case in two experiments on subject 3 and 1 on subject 4. The experiment therefore charted in figure 4 represents the only exception to this immediate increase in urea excretion in the six experiments of this type in which urea estimations were made. The more typical response is shown in figure 5 which represents an experiment on subject 3, who gave results apparently little affected by changes in room temperature. In a control experiment on this subject without lying down but taking the regular hourly meal (except for one accidental omission at 10 a.m.) the urea excretion remained between 13.2 and 11.3 millimols per hour from 12:30 a.m. to 9:25 a.m., between this time and 11 a.m. fell to 4.2 and between 11 a.m. and 12:30 p.m. to 4.0 millimols per hour. (In this subject samples had to be taken only every 90 minutes owing to the small volumes of urine excreted, see fig. 5.) In contrast with this it will be seen that on lying down in the experiment charted in figure 5 the urea excretion rose from 2.2 to 10.2 millimols per hour. In another similar experiment on subject 3, the urea excretion was 4.4 millimols per hour during the night, 4.8 in the first hour after waking, 6.4 in the next two hours and then rose to 8.7 on lying down. In subject 4 a control experiment gave a maximum urea excretion while moving about the laboratory of 10.9 millimols per hour, and in a similar experiment but lying down gave a maximum of 25.8 per hour. The effect of lying down on the urea excretion of subject 1 is seen in figure 8.

These figures demonstrate quite definitely an increase in the excretion of both water, chlorides and urea as the result of lying down; the increase in the urea was often very great, and in 4 out of the 6 experiments there was even an increase in the percentage strength of the urea in spite of the increased volume of urine. It cannot therefore be a simple result of the increased volume. In considering the diuresis caused by baths, the mere change from a standing to a lying down position has therefore to be taken into account.

EFFECT OF BATHS: *Partial immersion and urea excretion.* That the diuresis produced by baths is not dependent on the temperature has already been shown, and experiments with partial immersion demonstrate clearly that the diuresis follows immersion of the trunk, and is not produced by immersion of the limbs and also that only a part of the diuresis can be explained by the lying down position. This may be seen clearly in figures 5 and 6. Figure 5 represents a single experiment showing that

immersion of the body up to the level of 1 inch above the umbilicus produced no diuresis but that the increased secretion produced by lying down began to fall off; on the other hand complete immersion up to the neck produced a considerable diuresis with a further increase in the total chloride

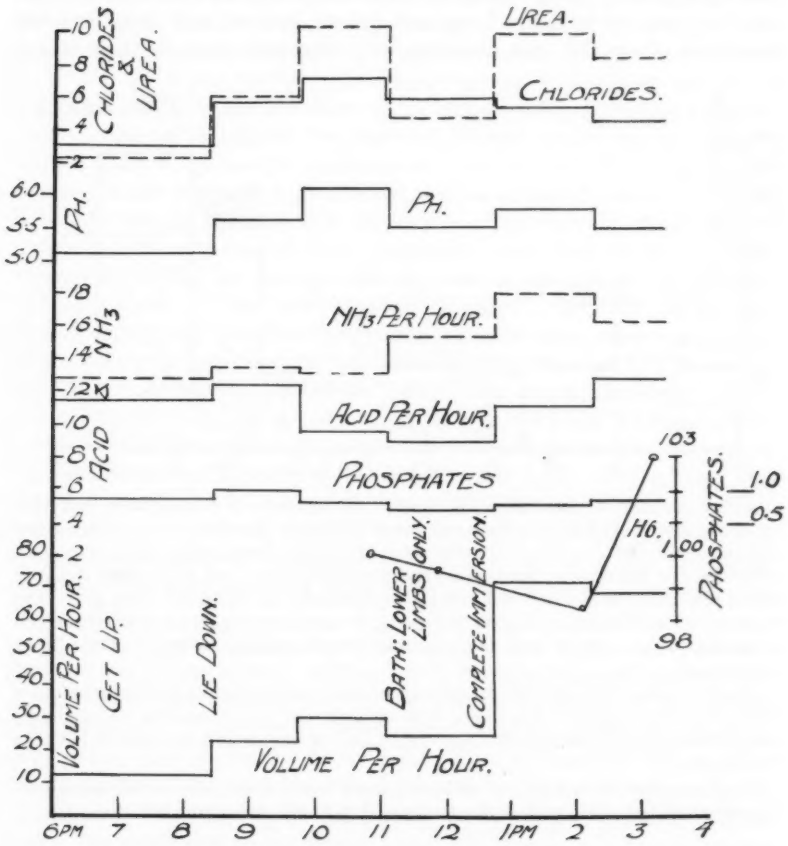

Fig. 5. Subject 3, August 24, 1923. Immersion by stages. pH values without loss of CO_2 except first sample. Lying down 8:30 to 11:08. In bath with water 1 inch above umbilicus 11:08 to 12:43—after this complete immersion. No appreciable change in blood pressure. Room temperature 17.7 but subject did not feel cold.

and urea excretion. Figure 6 shows the volume of urine excreted on a control day compared with that on two occasions when complete immersion was preceded by partial immersion. A diuresis similar to that with complete immersion has also been found to occur in subject 2 if the ab-

domen and thorax were immersed, but the lower limbs were kept out of the water, resting over the lower end of the bath. The essential factor appears to be the immersion of the trunk, and it is noticeable that the diuresis is associated with a considerable rise in the urea excretion, very similar to that produced by lying down. The increase in urea seen in the experiment represented in figure 5 was not unique, but was seen in all bath experiments where the urea excretion was estimated, even in those where

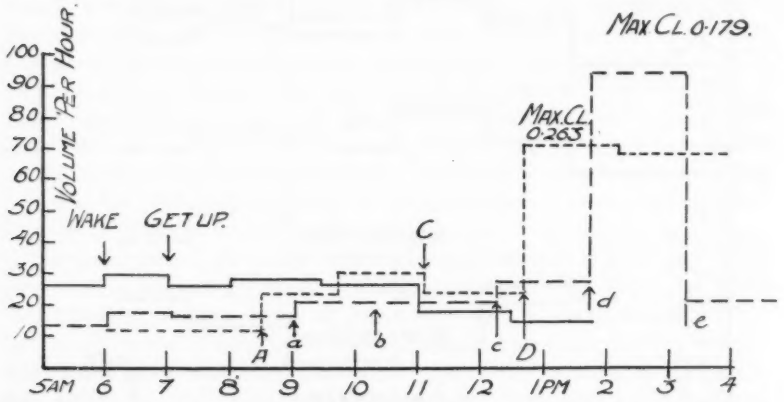

Fig. 6. Subject 3. Immersion by stages. Comparison of rate of urine secretion on November 25 with hourly meal but not lying down, charted———, and that on August 20 charted. — — —. On this date subject lay down from *a* to *b*, sat in bath with water to the level of the symphysis pubes from *b* to *c*, sat with water to 1 inch above umbilicus from *c* to *d*, was completely immersed at *d* and left bath at *e*. The secretion rate obtained on August 24 (see fig. 5) is included again for comparison and is charted ------; *A*, *C* and *D* correspond to conditions *a*, *c* and *d* of the other experiment.

On November 25 the maximum chloride concentration reached (in the preliminary control period) was 0.176 N between 9:25 at 11:00 a.m. On August 20 the maximum reached was 0.179 N between 10:17 and 12:15, and on August 24 it was 0.263 N between 7 and 8:35.

On November 25 and August 20 hourly meal from 7 a.m., but 10 a.m. meal accidentally omitted on November 25. On August 24 hourly meal from 8:20.

a preliminary period of lying down had already caused a considerable increase in urea excretion. The bath therefore always produced a similar effect to that seen on lying down, but one of greater intensity, and one induced even at a time when the lying down effect was subsiding. With the great diuresis caused by baths the urea percentage usually fell, so that the increased excretion might be explained as a simple washing out effect. On two occasions however, there has been a maintenance of the urea percentage level or even an increase in the percentage in spite of

the great increase in the volume of urine, suggesting strongly that the effect is not dependent simply on the volume of urine excreted.

Chlorides and water. The experiments plotted in figures 2, 5 and 8 show the effect of the diuresis on the chloride output. It will be seen that this is always increased, but much less than the water so that there is always a great fall in the percentage strength. The concentration of chloride at the height of the bath diuresis has varied between 0.024 N and 0.113 N. As a rule however the concentration at this time was about 0.03 N or 0.04 N. The condition of the subject seemed to make a considerable difference as regards the degree of diuresis, which varied considerably in apparently similar experiments. One factor influencing the degree of diuresis appeared to be the salt and water balance of the individual. It will be seen in figure 6 that the degree of diuresis on subject 3 in 2 similar experiments varied quite appreciably and that the higher level was reached on the day during which the chloride concentration of the urine had been lower in the control period. Figure 7 represents the diuresis seen in 3 complete immersion experiments on the same subject; again the greatest diuresis is seen on the day when the control urine had the lowest chloride concentration. It will be noticed that in all 5 experiments the rate of urinary secretion in the control periods was fairly constant, and it is evident that the greatest rate of urine secretion reached is lower when complete immersion is preceded by a considerable period of lying down and partial immersion. In subject 2 it was similarly found that the effect produced by lying down followed by immersion in stages gave a lower maximum rate than did the simple bath, and in this case also the degree of diuresis was approximately predictable from the previous chloride concentrations.

The bath diuresis begins to subside in an hour or two and generally gradually returns to normal, as has already been pointed out in the earlier paper. This return to normal may be difficult to detect if the subject (e.g., subject 3) is put onto a régime which necessitates his taking either more or less than his normal fluid intake, but in any case the effect of the bath is probably over in a few hours. It will be noticed that in subject 3 (experiments 2 and 3 of fig. 7) the diuresis may continue for some six hours under the conditions of the hourly meal, the volume of urine exceeding the fluid taken throughout this time. Usually however the urine secretion has approached or reached a normal level in from two to four hours. The total secretion of urine during this period over and above that which would have been formed if the rate of urine secretion had remained unchanged cannot be accurately estimated, but has generally been between 300 and 800 cc.

This diuresis may be diminished but not abolished by deprivation of food and water. The minimum bath diuresis in subject 1 (169 cc.

per hour) was seen when a bath was taken at 10 a.m., twelve hours after the last food or drink. This experiment is plotted in figure 8. Similarly subject 2 was deprived of food and water for 18 hours (from 8 p.m.) except

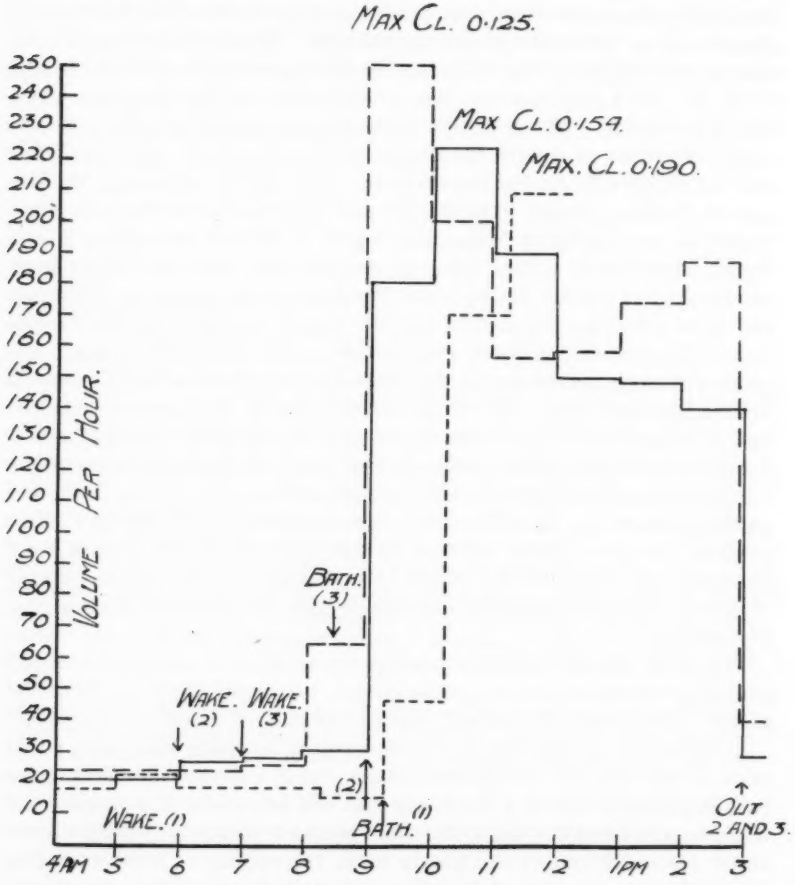

Fig. 7. Subject 3. Further evidence of effect of chloride and water balance on rate of diuresis. Experiment 1 charted - - - - - - - July 20, 1923. Hourly meal from 6 a.m. Maximum chloride concentration in preliminary period 0.190 N between 6 and 8:15.

Experiment 2 charted ———. July 27. Hourly meal from 7 a.m. Maximum chloride of preliminary period 0.154 N between 8 and 9 a.m.

Experiment 3. Charted — — — August 3. Hourly meal from 7 a.m. Maximum concentration of chloride in preliminary period 0.125 N between 7 and 8 a.m.

for 100 cc. of water taken at 10 p.m. and 100 cc. of water and ½ slice of bread and water at 8 a.m.; from 11:40 a.m. to 1 p.m. urine was secreted at 42 cc. per hour, lying down from 1 a.m. to 2 p.m. 84 cc. of urine was

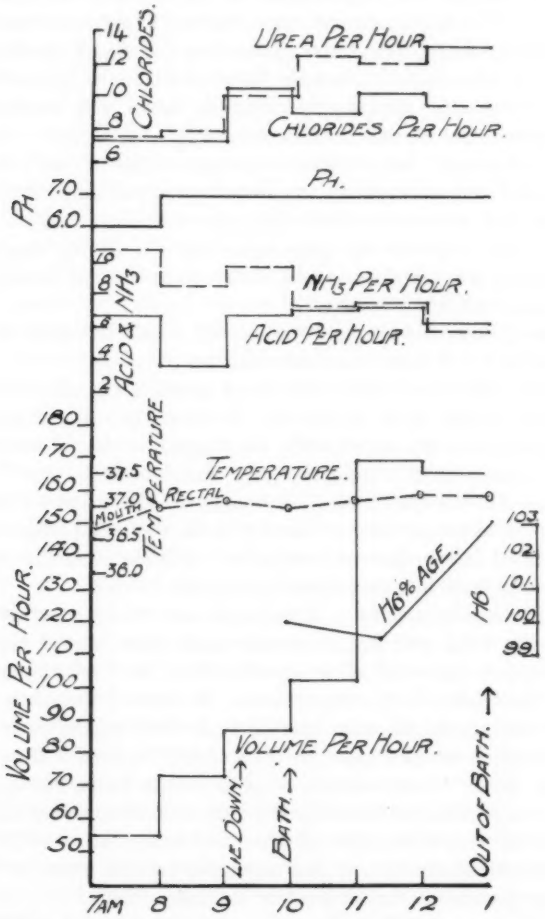

Fig. 8. Subject 1, November 25, 1923. Effect of bath taken without hourly meal and with no food or drink for previous 12 hours.

passed and in a neutral bath from 2 p.m. till 3:15 p.m. the rate rose to 96 cc. per hour; this was the lowest bath diuresis ever seen in this subject. But the volume is not proportional to the proximity of recent meals; thus subject 1 took a bath at 10:15 a.m., no food or drink having been allowed since

the previous night except for 1 glass of water and a slice of bread and butter at 7 a.m. and a maximum rate of 244 cc. per hour was recorded; while on the hourly diet of bread and water started several hours before the bath and continued throughout the experiment the highest rate reached was only 300 cc. Again the next to lowest rate observed in subject 2 was in an experiment where the bath included immersion by stages starting at 1:25 p.m., a normal breakfast having been taken at 7:45 a.m. (the chloride concentration of the urine before the bath was taken, was however on this day much above the normal for this subject).

Possibly the diuresis may be absent in some subjects. In 2 experiments on one subject out of 14 used, no diuresis was evident, but the control rates of secretion were not exactly determined for this subject. The conditions for both experiments were abnormal—in one no food had been taken for about fourteen hours and in the other he had taken a normal breakfast some two hours earlier.

The degree of diuresis attained varies then somewhat with the previous intake of fluids but this factor is not dominant.

Phosphates. Only two charts are given showing the effect of the baths on phosphate output (figs. 2 and 5). In these two experiments and in several others there was practically no change in the phosphate output per hour. Occasionally a slight increase was found.

pH values. All the charts show a change of the urine to the alkaline side during the diuresis; figures 2 and 8 show the usual effect, the actual values obtained being close to neutrality. Figure 5 shows an example of one of the most acid urines found in a bath diuresis.

Acid and ammonia excretion. The charts show the inconstant and slight changes in the total acid and ammonia excreted in neutral baths. Thus figure 2 shows a slight fall after a preliminary rise even though this subject had a slight fall of body temperature. In figure 5 there is a rise, and in figure 8 no change in the acid excretion; in both experiments there was no change in body temperature in excess of 0.1°C., though if anything the temperature rose. There can be no doubt that baths raising the body temperature diminish considerably the acid excretion, as has already been pointed out (2) but with baths at a neutral temperature either slight increases or decreases of the acid and ammonia excretion may be found, the result being apparently not dependent on temperature.

Hemoglobin changes. These have been determined on venous blood taken from an arm vein without stasis, and the hemoglobin value taken when the subject has been lying down for at least 20 minutes (and usually 45 minutes) before the bath has been used as the standard. Usually two samples of blood have been taken at the same time and have been read independently in the colorimeter. The differences found between these two samples rarely amounted to more than 0.7 per cent and usually did not exceed

0.5 per cent; even if the blood was taken from separate venous punctures at short intervals the difference between the two samples was usually no greater. Probably the figures may be considered accurate at least to the nearest whole number. In all a series of hemoglobin readings was made once each on subjects 1 and 3 and 3 times on subject 2.

Subject 2 was similarly numbered in a previous paper on diurnal variations in hemoglobin (9) and it will be seen that between 10 a.m. and 4 p.m. swings of only about 2 per cent were seen. Under the conditions of the bath experiments, lying down and with either constant meals or complete starvation, the hemoglobin changes have been more regular. In all experiments on this subject (one of which is plotted in an earlier paper (3)) a slight concentration of the blood occurred following complete immersion in the bath. Often the amount has been so small as to be only slightly exceeding the experimental error, but if the effect were not real, one would expect the changes to vary in direction as well as degree. There seems to be no doubt that in this subject there is always a slight concentration of the blood, which becomes greater if the bath is hot and the subject sweats. Such a concentration is one relative to the lying down normal, for in this subject the lying down figure has in every case (4 experiments), been somewhat more dilute than that taken just before lying down (compare Dreyer, Bazett and Pierce, figs. 1 and 2). On one occasion this subject lay down from 8:30 a.m. to 9:40 a.m. then took a normal blood sample and after this sat in the bath till 10:30 with water at 36.9° to the level of the umbilicus when the hemoglobin value dropped to 97.8 per cent; from 10:52 till 11:27 he was completely immersed to the neck in water at 36.5, a temperature sufficiently high to cause a slight rise in body temperature and his hemoglobin value rose again to 100.8 per cent. He remained in the bath till 1:35 p.m. when the hemoglobin percentage was 103.8 per cent. Thus without complete immersion there was a dilution in the warm bath, corresponding closely with Barbour's results on dogs (1), while with complete immersion this became a concentration in spite of the rise in temperature and without appreciable sweating. The initial dilution might belong to the morning changes in this subject, but as he rose at 6:45 a.m., it is unlikely. On this subject the outstanding result is the extraordinary consistency of a slight blood concentration on being immersed in a bath to the neck, in spite of the normal extreme variableness of the hemoglobin percentages; this concentration in the bath was seen whether the subject took the bath about 10 a.m. when taking hourly meals including 100 cc. of water or whether he took the bath at mid-day without food but after a normal breakfast.

From figures 5 and 8 it will be seen that two other subjects both gave an initial dilution followed by a concentration, the initial dilution occurring even with complete immersion.

All the blood examinations therefore show an ultimate slight concentration, with an initial slight dilution, which in subject 2 was only seen with partial immersion.

Discussion of results: *Waking and sleeping tides.* The figures given show quite clearly the presence of an alkaline tide in the morning, quite apart from the effects of meals, if this be estimated by titratable acidity per hour. Those workers who have denied the waking alkaline tide have often worked solely by a pH method (14) which gives much less striking results, and they may by chance have used subjects in which an alkaline tide is slight or even absent. This alkaline tide does not appear to have a similar origin to the alkaline urine induced by baths which raise body temperature, since the waking alkaline tide may occur without a measurable body temperature rise.

Also on waking and with a course often parallel to that of the akaline tide there is a chloride tide, of which there has already been evidence in previous literature. This chloride tide is clearly seen also in Simpson's work (25), his chart 2 giving an excellent example; in his experiments as in many others the giving of water on waking has made it impossible to decide if the chloride increase is secondary to the increased water output. In our experiments the chloride output has increased often with an enormous increase in the chloride concentration, thus excluding such a hypothesis. This chloride tide varies in degree with different subjects and appears to bear some relationship to the alkaline tide and possibly to body temperature changes, but has been observed even when there was no change in body temperature detected, nor any alkaline tide in the urine. It is not possible to determine its origin from the data available nor need the absence of a urinary alkaline tide necessarily indicate the absence of one in the blood—thus for instance, subject 2 was usually in the morning excreting urine of about the extreme limit of known pH values for urine (4.8 or 4.9) (30) and the amount of acid excreted may have been limited by the kidney rather than by the blood condition.

If Leathes is right in his deduction that the blood is more acid during sleep owing to a depression of the respiratory center, the chloride tide might be due to a chloride shift secondary to the change in reaction. If the differences between arterial blood when asleep and that when awake are as great as those betwen venous and arterial blood (the figures given by Endres suggest a fall of 8 to 10 mm. in alveolar CO_2 tension on waking, or even a greater change, and Leathes' figures are not much lower) the observations of Van Slyke, Wu and McLean (27) suggest the shift of 1.1 to 1.4 milli-equivalents of Cl per liter of blood from the corpuscles to the plasma, or from 4 to 7 milli-equivalents of Cl for an adult. In subjects where the chloride tide is marked the excess of Cl excreted in the morning amounts to 3 to 10 milli-equivalents, the total excess being impossible to determine

accurately from our data as the chloride excretion at other times of the day is somewhat inconstant. It is therefore conceivable that the extra excretion of chloride is dependent on a chloride readjustment in the blood alone, if the changes are of this magnitude. A more probable explanation would seem to be one assuming a chloride shift not limited to the red blood corpuscles, but involving tissue cells as well, a reaction which has already been considered possible by the above authors (27).

With the chloride tide there is apparently a tendency to phosphate retention—a fact already suggested by Kleitman's work. Additional evidence of such an interdependence is found in the observations of Rockemann (21), where the administration of NaH_2PO_4 was found to cause some chloride retention. There seems to be little doubt that the chloride and phosphate tides are to some extent related.

It is indeed probable that very complicated interactions of different parts of the body are concerned in the various diurnal changes and that none of these are completely independent of the others. There is at any rate some similarity to be seen between the curves for diurnal variations in body temperature and excretion of acid, phosphates, chlorides in our experiments, and of water (25), in alveolar air (10), (16), in hemoblobin percentage (9) and also in the size of the red blood corpuscles (21), even though the relationship is in some cases an inverse one.

The bath diuresis. As has been pointed out, this shows considerable similarities with the diuresis seen on lying down; in both the volume is raised, the chlorides being also raised, but diminished in concentration, while the urea excretion is considerably increased; in both the diuresis is accompanied by a fall in blood pressure; but in the bath there is a concentration of the hemoglobin, while, when lying down (in one subject at least) there is a dilution. The most probable hypothesis for the increased urine formation when lying down would appear to be a dilatation of the splanchnic vessels rendered possible by the cessation of the vertical position, altering renal circulation or improving intestinal absorption; if the latter, the nitrogenous contents of the intestine might explain the raised urea excretion. Similar factors might well be involved in the still greater effect produced by a bath.

The increased urine cannot be derived to any extent from the blood, since it is much too great considering the small changes in hemoglobin; our previous hypothesis of tissue fluid (2) is not supported by the absence of any effect of immersion of the lower limbs alone, while the relative importance of the immersion of the trunk suggests for the most part an intestinal or renal origin. There can be no doubt that in a bath venous pressure is raised in the lower part of the body, and a rise even in the veins of the upper part of the body has been demonstrated by Schott (24). The actual pressure in the veins submerged in the water is hard to deter-

mine, but that in the veins of the foot is certainly above 25 cm. of water, since they do not collapse in water of this depth and a rise in pressure in the abdominal veins would also appear inevitable. On the other hand, the intrapleural pressure is probably much less altered and the effects produced in the venous circulation must resemble those described by Thorington and Schmidt (26).

The factors concerned are too complicated for present analysis; the water pressure exerted on the relaxed abdomen must raise the intra-intestinal pressure but presumably it should affect the vascular system to a similar extent. On the other hand a raised venous pressure has been shown by Richards and Plant (22) to produce an increased flow of urine even in the eviscerated animal, and though Thorington and Schmidt found that a high intra-abdominal pressure diminished urine secretion they dealt mainly with rises two or three times as great as those which would result from the bath. A slight rise of venous pressure might affect either glomerular filtration or tubular reabsorption, causing an increased volume of urine and if this caused a slight concentration of the blood, there might be a considerable absorption of intestinal contents in readjusting to a new osmotic balance. According to McLendon (18) and his co-workers, the reaction of the intestinal contents is very variable and may often be quite acid, and such variations may play a part in determining the variable effect of a bath on the acid excretion and pH of the urine. Such a tentative hypothesis, however, merely indicates the necessity for further analytical work.

It is somewhat remarkable that in the experiment plotted in figure 8, where the subject was deprived of water, the subject complained of thirst in the bath, in spite of showing a marked diuresis.

Hemoglobin changes. A tentative hypothesis may be advanced that a drop in hemoglobin percentages occurs in the reaction to the rise of skin temperature produced by the bath, which would bring the results into agreement with Barbour's, and that this tendency is opposed and eventually overcome by some other action of the bath which causes a concentration.

CONCLUSIONS

1. In many subjects a urinary alkaline tide is seen on waking even when no food is taken and the subject remains in bed.

2. On waking there is also nearly always a chloride tide; this is often accompanied by an actual increase in the chloride concentration of the urine.

3. Some partial interdependence of alkaline, chloride and phosphate tides is suggested by the results obtained. These do not appear to be caused by changes in body temperature though possibly affected by them.

4. Lying down causes an increase in the urine secreted per hour, with a considerable rise in the urea excreted and often a rise in the urea concentration. Increased intestinal absorption is suggested as a possible cause.

5. Immersion in baths of neutral temperature causes the excretion of a large volume of urine consisting mainly of water in which the chlorides drop to about 0.04 N, though the total excretion of chloride per hour is increased. The urea concentration however often drops very little so that the urea output per hour is much increased. Even if the subject has been lying down for some time previously so that the effect of this is passing off, the urea excretion may be much raised by the bath. The phosphate excretion is practically unchanged.

6. The bath diuresis is almost invariably seen, but varies in degree in different subjects and in the same subject on different occasions. The degree of diuresis reached in any subject has proved to be less the greater the chloride concentration of the urine in the preliminary control period. No constant relationship has been seen between the rates of urine formation before and in the bath.

The diuresis usually subsides in a few hours after the excretion of some 300 to 800 cc. of excess fluid.

7. The diuresis is induced by immersion of the trunk in water without the limbs being immersed but is not caused by immersion of the lower half of the body up to the level of the umbilicus.

8. The baths when entered cause a sensation of warmth. The hemoglobin percentage has fallen slightly on entering the bath in some subjects but later in all subjects it has shown a concentration.

9. It is tentatively suggested that the pressure of the water on the abdomen raises venous pressure, and that in adjusting to this condition, new salt and water balances are set up, causing secretion of urine and absorption of fluid. The maintained high urea percentage of the urine is taken as evidence that the main source of this fluid may be the intestine.

It is a pleasure to express our thanks to other students in this school, who aided this work by making preliminary less detailed experiments, and to our technician, Mr. A. Afford, for this skilful assistance in taking blood samples from and controlling the experiments on subject H. C. B.

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